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Proceedings of the Seventeenth Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, B.C. Vol. 1

Comptes rendus du dix-septième colloque annuel sur la toxicologie aquatique : 5-7 novembre 1990, Vancouver, (C.-B.) vol. 1

Editors/Éditeurs

P. Chapman, F. Bishay, E. Power, K. Hall,
L. Harding, D. McLeay, M. Nassichuk and/et W. Knapp

February 1991

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Rapport technique canadien des sciences halieutiques et aquatiques n° 1774 (vol. 1)

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PREFACE/PREFACE

The 17th Annual Aquatic Toxicity Workshop was held at the Hotel Vancouver in Vancouver, B.C. on November 5-7, 1990. The theme of the 1990 Workshop was "Threshold Biological Response: Predicting and Determining Ecological Relevance".

The Seventeenth Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the proceedings are published with the support of the Department of Fisheries and Oceans.

The Workshop included 6 plenary presentations, 116 platform presentations, 27 workshop presentations, 25 papers in poster sessions, and several panel discussions. Eight different workshops were held with summaries presented at a final plenary session. Total attendance was 439.

Le 17^{e} colloque annuel sur la toxicologie aquatique a eu lieu les 5, 6 et 7 novembre 1990 à l'Hôtel Vancouver de Vancouver (C.-B.). Le thème choisi pour le colloque de 1990 était : "Le seuil de réponse biologique : son importance pour l'environnement."

Le 17^{e} colloque annuel sur la toxicologie aquatique a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces colloques annuels organisés par un Comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec l'aide du ministère des Pêches et Océans.

Le colloques a donné lieu à 6 communications lors de séances plénières, 116 exposés d'invités d'honneur, 27 exposés en ateliers, 25 communications par affichage et plusieurs panels-discussions. Les résumés des huit ateliers ont été présentés à la séance plénière finale. 439 personnes ont assisté au colloque.

EDITORS' COMMENTS/ REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and a list of participants are also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur; ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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Many persons worked to make the workshop a success. We extend our thanks to all of them, in particular to the volunteers, authors, session chairpersons and the participants for their contributions. We also extend our gratitude to the staff of E.V.S. Consultants and of the Hotel Vancouver, Vancouver, B.C., for their co-operation and efforts for the workshop.

Le comité d'organisation tient à remercier les commanditaires de leur soutien financier ou autre.

Nos remerciements vont aussi à toutes les personnes qui ont contribué au succès de colloque, en particulier aux travailleurs bénévoles, aux auteurs, aux présidents des diverses séances et aux conférenciers qui ont présenté leurs travaux. Enfin, nous voulons exprimer toute notre gratitude au personnel de E.V.S. Consultants et de l'Hôtel Vancouver, de Vancouver (C.-B.), pour leur collaboration et leur excellent travail lors du colloque.

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BANQUET SPEECHES

COSTS OF ENVIRONMENTALISM

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The Vancouver Aquarium emphasizes basic research on natural history, at the descriptive level. Toxicologists, on the other hand, primarily conduct applied research, but, as with the Aquarium's research, toxicological studies are also primarily at the descriptive level. Therefore, we share a common ground, establishing facts, at a time when the trend, especially in academia, is toward the analytic level of science. Our emphasis on fact may give us a common perception of environmentalism and the way in which activists and the media deal with factual information.

I will define environmentalism in the context of activism generally, then speak about my experience in the context of Howe Sound. Howe Sound is the location of most of my field research, as well as a site of environmental concern over pulp mills and dioxins. The costs of environmentalism will be considered in terms of direct financial costs versus broad implications.

These days, activism has to be defined with respect to the news media. It is often said that one should avoid a direct confrontation with an activist in front of the media, because their righteousness will overwhelm your rationality. Activists have absolute conviction, are self-appointed, and typically lack credentials. This is not to say that they lack professionalism. They are highly professional in terms of fund-raising by means of media campaigns. They use Machiavellian tactics; the end justifies the means as far as raising public awareness. Thus even highly reputed organizations which rely on public appeals for their international conservation activities will make exaggerated predictions of extinction rates in tropical rainforests, based on guesses at insect diversity, even though their own efforts and expertise center on endangered plants or vertebrates, rather than insects.

This interaction between awareness of issues and fund-raising can be exploited in an extortionist fashion. An example involves two experiences I had in different years with the tactics of door-to-door canvassers for Greenpeace. Both times, when I declined to offer a contribution toward "saving the whales" I received the rejoinder that I therefore "must not care about whether the whales are killed." Most people who encounter this tactic are not aware that Greenpeace employs professional canvassers who pocket one third of their take. With professional activists, concern for molding attitudes may be secondary to concern for funding their jobs.

Environmental activism is a growth industry in terms of income, but that income involves costs. Media coverage of activists' publicity stunts generates donations to their organizations. There is a cost to society regarding the direction of cash flow in small-scale philanthropic giving and the merits of the different causes. Furthermore, some philanthropic organizations keep costs of fund-raising below 25% of the monies received, and others run at over 50%, whereas activist organizations which maintain that their media events are their means for creating public awareness are in fact rationalizing a fund-raising cost of 100%. That is, the funds go full circle into further media events, with payroll and other expenses taking up all the donations. The ultimate cost resulting from slanted activist media coverage and its impact on public opinion is that it leads to over-simplification, polarization and crisis-orientation.

Activist campaigns have target groups which do not encompass the public as a whole. Next to environmental activists themselves, there are people enthusiastic about environmental issues, but unsupportive of radical change. They may therefore be willing to donate money although they will not become personally involved in the campaign. Others tend to believe that society and government are successfully resolving these issues, and so are less likely to contribute. Others are even more unlikely to give for a variety of reasons ranging from poverty to cynicism over prospects for solutions to refusal to believe that environmental threats are real. Environmentalists are largely preaching to a converted minority.

Environmentalists have focussed on Howe Sound, near Vancouver, since the early 1980s when strip mining was briefly attempted, but curtailed because of production costs. Since the discovery of dioxins, their focus has been on pulp mills, Dioxins are proclaimed by the activists, and then the media, as the deadliest of toxins, yet dioxins are suspected of being cancer promoters in humans, not carcinogens, and not direct metabolic toxins. Greenpeace launched a media campaign against the mills, based on dioxins, aiming at compliance to regulations. The compliance issue involves managerial and political considerations of economic costs and benefits which extend beyond the present issue of activism. The media coverage of dioxins from pulp mills has slackened, so that Greenpeace just recently came back with a report of dioxins in mother's milk, but the coverage has quickly centered on benefits of mother's milk and the potential cost to society of scaring women into avoiding breast-feeding their babies. This slant will probably not be pursued because of its lack of fund-raising potential.

Late in 1989 I spent a considerable length of time being interviewed by The Province, a local newspaper, on my research in Howe Sound. I made only equivocal statements in answer to questions on environmental issues; emphasizing how little we can correctly document. No story was run. I then scheduled a field trip and interview with the other daily, The Sun, on the same subject. Just prior to that interview, however, a newspaper-style flyer was distributed by the Environmental Watch/Western Canada Wilderness Commitee, entitled "Stop the Killing of Howe Sound NOW." That flyer was a topic of conversation during the field trip and the resulting article was rewritten by the city editor to make it as provocative as possible, emphasizing the abundance of marine life in Howe Sound. The simplification process by the media seems to preclude any middle ground.

The relevance of this sort of distortion towards extreme viewpoints brings up the subject of the cost of crisis-orientation. With regard to dioxins, beyond the human cancer threat and the known effects of dioxins in fresh water, there seems to be very little established regarding possible toxicity of dioxins to marine life in Howe Sound. In addition to the cost to the fishery of closures for prawns and crabs due to dioxin content, there is the enormous industrial cost of the mills switching to chlorine dioxide substitution. This switch involves the fairly radical experiment of eliminating chlorine breakdown residues, in the absence of documented toxicity to marine life, at the potential cost of introducing all of the breakdown residues of chlorine dioxide, which may prove to have different impacts on marine life. A further cost results from the undermining effect of the activists' portrayal of pollution as limitless and pervasive. I have encountered the suggestion that all of my natural history research in Howe Sound may involve artifacts caused by pulp mill pollution, an idea which is not borne out by my efforts in more remote areas. There is also the cost to taxpayers of allocating tax dollars to predictive analytic modelling of dioxin transfer in Howe Sound when none of the necessary metabolic parameters for that model have been estimated, much less measured, for any animal species in Howe Sound. Crisis-orientation has resulted in the effort to achieve solutions at once, without any background documentary work. We must reject crisis-orientation; we must obtain the correct data at the descriptive level. We must either take the required time or spin our wheels.

Vancouver Aquarium research constitutes the only institutional commitment to a continuous research presence in Howe Sound, providing the only chance to document changes which may occur over time. This work is funded by tourist dollars earned by a non-profit organization, with no supplementary taxpayer support and no public appeals. Canada has no Smithsonian Institution to undertake natural history research, yet Canada is not a third world nation, and thus cannot expect other countries to conduct its baseline floral and faunal surveys. Canada has a natural resource economy without basic natural history research to provide for management of all its resources.

Vancouver Aquarium research in Howe Sound has included documentation of nursery gounds of hake and prawns, lack of planktonic dispersal of shoreline fishes, variability in larval fishes and new taxonomic descriptions of larval fishes. More recently, with the recognition that early life history stages are most susceptible to interannual variations due to weather effects, an additional focus has been placed on prominent, long-lived indicator species. On deep fjord walls, basic studies on growth of glass sponges will also provide for long-term data records of a major faunal component. Similarly, on rocky shores, study of the seasonal movements of the purple starfish will provide for continuous records of an intertidal keystone species. Both the sponges and the starfish have temporal stability and certainty of access which will provide balance, in terms of monitoring, to the studies of larval stages.

The Aquarium's commitment is conservative, yet optimistic. We place faith in the resilience of nature and that there is time for long-term commitment to documenting necessary facts. This commitment has a place which is in contradistinction to environmental activism.

SUZUKI MEETS GODZILLA -- A TALE OF CANADIAN EFFLUENT REGULATIONS

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ABSTRACT. An overall strategy of regulation should always include three tactics, with the most restrictive one in a particular situation being operational: (1) technology-based limits derived from good industrial practice, to be applied at the end of the effluent pipe; (2) limits based on water quality criteria, strict enough to eliminate sublethal effects in the receiving water beyond a specified mixing zone; and (3) periodic ecological surveys to check the effectiveness of the first two approaches.

"Levels of Achievement" could also be formulated to provide increasingly restrictive steps for management. The final level should represent an ultimate goal of eliminating deleterious discharges, i.e. the effluent-free industrial operation.

Pulp and paper mills provide examples. Recent Australian guidelines for kraft mills give a balanced blend of all three tactics. Federal requirements in U.S.A. also have the three tactics, with good water quality limits for sublethal toxicity. Existing and proposed Canadian federal regulations have many deficiencies. First, they continue to focus on tactic no. 1, the technology-based limits, and fail to include water quality limits to account for differences in waterbodies. Second, Canadian administrators refuse to consider *amount* of toxicity discharged, using instead the concentration-based toxicity of effluents, and thus failing to encourage water conservation. That is one example of the general failure to adopt an ecosystem/biosphere stance. Third, Environment Canada is apparently unable to grasp the purpose of biological monitoring of downstream communities to check the effectiveness of regulation. Partial compensation for deficiencies is provided by most provinces, which may add water quality limits in one form or another, and may provide excellent river surveys.

This paper was delivered as a speech at the Workshop banquet. In the first section of the talk, Dr. David Suzuki was used as a personification of the forces of goodness at work for better environmental legislation. Dr. Suzuki has tried to convince us to embrace an ecosystem/biosphere approach. He has warned that saving the planet requires that we adopt rapid and radical changes in our approach.

Godzilla, the resurrected giant dinosaur of late-night movies, was used as a personification of the forces of environmental ignorance, destruction and inability to adapt to changed circumstances. For the purpose of considering regulations against water pollution, the role of Godzilla was assigned to Ottawa-based upper and middle management of Environment Canada.

PHILOSOPHICAL OBJECTIVES OF REGULATIONS

The reason for regulating industrial water pollution is to protect natural communities of organisms. If done, that will include protection of humans as part of the general ecosystem. The ideal and ultimate goal must be no discharge of effluent, since plants and animals in the natural communities are adapted through millennia to conditions *without* human industrial input. On the other hand humans are now part of the system and cannot live without producing some waste. Hence, any regulation of industrial discharges will always be a compromise between the ideal and whatever is possible at the moment. Regulations should be considered as temporary resting-places on the road to a goal of zero discharge.

Effluents from pulp and paper mills are used as examples in this paper. The proposals offered are neither radical nor new, but I have been surprised how many people dealing with effluent regulation have neglected to contemplate basic principles and methods of applying them.

THREE TACTICS WITHIN A STRATEGY

When designing regulations, three tactical approaches should always be used within a general strategy, since no single approach is completely satisfactory (Table 1).

(1) Limits based on good current industrial technology, controlling amounts of pollutants to be emitted (in general, the "Best Technology" approach).
(2) Limits based on water quality criteria, to prevent sublethal effects in aquatic communities.
(3) Periodic biological surveys to check that the first two tactics give the protection intended.

Table 1. The three elements that should be included in regulations for discharges.

CONTROL TACTIC AND ITEMS CONTROLLED		
(At end of the pipe)	(At edge of the mixing zone, and beyond)	
(1) Technology-based	(2) Water-quality-based	(3) Check by ecological survey
Numerical limits for specified variables and toxicity	Numerical limits for specified variables and sublethal toxicity	Limit(s) for degree of change in community.
a, b, c	j, k, l	x, y, z

TACTIC 1. TECHNOLOGY-BASED LIMITS

The objective of this tactic is to achieve effluent discharges that represent good industrial practice; the approach should be used to find and remedy any grossly unsatisfactory effluents. This tactic is required for an eventual no-discharge society. The degree of control exercised at source should be reasonable for today's technology, even if there is great dilution at a given site. The discharge limits are dependent on the current status of industrial processes, so we can include in this approach, the concepts intended by the internally conflicting term "Best Available Technology Economically Achievable" (BATEA) or more simply "BAT".

These limits would be applied at the point of discharge, or "end of the pipe" and such effluent limits have been commonly used by governments during two decades. For example Canadian federal regulations for the pulp industry have required since 1971, non-lethality to fish at the end of the pipe, and pollutant loadings based on units of production (e.g. kg of total suspended solids (TSS) and BOD per tonne of pulp). The limits were set on the basis of good practice at that time.

Table 2. Items that might be regulated for pulp mills under tactic number one.

CONTROL TACTIC AND ITEMS CONTROLLED				
(1) Technology-based				
TSS kg/t	BOD kg/t	AOX kg/t	Persistent bioaccumulative toxicants *	Toxicity TEF as m^3/t
11 ?	7.5 ?	1.5 ? 2.5 ?	TCDD = no increase	50-150 ?
			Substance B = ?	
			Substance C = ?	

* This category allows limits for amounts of specific dangerous chemicals. The numerical limit would depend on the substance. AOX or EOX might be considered part of this category, as families containing bioaccumulative toxicants.

Obviously, there is no particular environmental logic in effluent limitations based on "good industrial practice", which will inevitably result in under-protection at some locations and over-protection at others. An effluent might have a very low BOD, yet be large in volume and cause low oxygen when discharged into a small river. Conversely, an effluent that killed trout might not cause observable effects on organisms in a large body of receiving water. Examples of both situations are easily found for pulp mill discharges.

Limits based on good practice should not generally be the strictest ones applied to a discharge, since by their nature they do not deal with ecological questions. Such limits should be of moderate strictness, sufficient to prevent obvious abuse. They should be considered as a coarse screen to bring each discharge to a reasonable level of performance by industry standards. On the other hand, good-practice limits might be the strictest ones in situations of great mixing and dilution, when tactic no. 2 (see next section) was not very restrictive.

A minimal list of items that might be regulated for pulp mills on the basis of industrial technology includes the old-fashioned items TSS, BOD, and fish lethality (Table 2). All these items should be regulated under tactic no. 1 in terms of *amounts* of that item which can be discharged per tonne of production of pulp/paper (kg/t or m^3/t in Table 2). That fits the goal of technology-based regulations, to achieve discharges representing good or superior industrial performance, i.e. the concept of BAT. A bigger mill would be allowed to discharge larger amounts of pollutants, which is why tactic no. 1 has no particular relation to ecosystem protection.

Toxicity Emission Factor

To an ecosystem, what matters about toxic pollutants is the *amount of toxicity* received. Toxicity should be limited in terms of the amount or "mass loading", within tactic no. 1. This important feature is exactly parallel to the limits for TSS and BOD as amounts per tonne of pulp. TEF stands for *Toxicity Emission Factor* and is a measure of "m^3 of just-lethal effluent" per tonne of pulp. TEF is calculated from volume of effluent and the median lethal concentration (LC50) for fish or other test organism:

$$\text{TEF} = \frac{\text{Rate of effluent discharge (as m}^3\text{/tonne of product)}}{\text{LC50 (as a decimal)}}$$

TEF should be used for regulation since it incorporates water consumption by the mill, and provides a positive framework for water conservation. Lower water use is generally linked to good mill design, good practice and fewer leaks and spills. If a mill used a lot of water and had a weak watery effluent, it might appear benign in a toxicity test based simply on concentration of effluent, although total amount of discharged toxic substance might be high. Conversely, a mill which reduced its use of water should not be penalized if *amount* of toxicity stayed the same, even if there were an apparent increase in concentration-based lethality.

One apparent difficulty is that no LC50 can be measured when it is above 100% effluent, and hence there is no real number for calculating TEF. The solution is to assume, for purposes of regulation, that >100% is 100%. Accordingly, if a status of non-lethality were attained, the TEF would be estimated as the effluent flow. That would tend to squeeze water use, i.e. encourage water conservation and recycling, which would be a desirable thing.

Persistent bioaccumulative toxicants

These make up the one category of pollutants that should always be strictly regulated under tactic no. 1, limiting the *amount discharged* rather than depending on a water quality approach (tactic no. 2). For such toxicants which travel through the world biosphere and accumulate in segments of it, no consideration should be given to characteristics of the site or available dilution. There are already too many examples of bioaccumulative toxicants which have travelled the world and accumulated in predatory animals, even in the Arctic.

TACTIC 2. LIMITS DEFINED BY WATER QUALITY OBJECTIVES

> *A river does not increase in size or in water flow every time a pulp mill increases its production rate. We want to see regulations that are based on the ecological constraints of the rivers.*
> Canadian Wildlife Federation, in a submission on the proposed pulp and paper regulations. (In *International Wildlife* Sept-Oct 1990, p. 25.)

The objective of this tactic should be to eliminate all ecosystem damage, beyond the edge of a specified mixing zone. This tactic is required because the limits of tactic no. 1 may not be good enough. The water quality standards of tactic no. 2 should often be the strictest part of the enforcement package. The need for tactic no. 2 is not difficult to appreciate, as indicated by the quotation starting this section.

Under the water-quality tactic, *location* of an industry is obviously of great importance, whether a tide-swept section of Atlantic Ocean or a slow-moving shallow river. That contrasts with the basic principle of tactic no. 1, which imposes the same standard regardless of location. With regulations on receiving water quality in place, industries would be discouraged from locating at places where they would discharge into a small or sensitive body of water.

This tactic would be based on sublethal toxicity tests as well as on chemical limits for certain items that have well-known, defined ecological effects and are easily measured. Examples of possible limits for pulp and paper mills are given in Table 3.

The toxicity requirement under tactic no. 2 could be as simple as "No sublethal effects measurable at the edge of the specified mixing zone" (last column of Table 3). That would be periodically assessed by short-term sublethal testing with a variety of tests such as algal production, reproduction of daphnids, and early life stages of a fish, as in U.S. practice (see section below). The concentration expected at the edge of the mixing zone would have to be at or below the no-observed-effect concentration (NOEC) found in the tests. Persistent bioaccumulative toxicants were covered under tactic no. 1.

Table 3. Variables that might be limited to defined maxima at the edge of a mixing zone. The zone would be delineated for each discharge location. The example is for pulp mills.

CONTROL TACTIC AND ITEMS CONTROLLED

(2) Water-quality-based (Edge of mixing-zone)

TSS mg/L	DO	AOX mg/L	Sublethal toxicity
≤ 25 ?	High level of protection *	2 ?	absent

*

Spring, Fall, Winter	70% saturation = minimum allowable DO
Summer	83%

(Simplified from Davis 1975)

The physico-chemical water quality limits for pulp mills could include concentrations of TSS and dissolved oxygen in the receiving water after dilution. The maximum of 25 mg/L for TSS shown in Table 3 is based on recommendations of the European Inland Fisheries Advisory Commission (Alabaster and Lloyd 1980) but a value of 10 mg/L might be used, following the suggestion of CCREM (1987). The oxygen level in Table 3 is a slightly simplified version of the "high level of protection" recommended for a mixed community of freshwater fish by Davis (1975) after he had reviewed the degree of impairment of performance by fish at various reductions of oxygen. Davis gave a choice of three levels of protection, following the principle established by Doudoroff and Shumway (1970).

A maximum concentration of Adsorbable Organic Halogen (AOX) has been shown in Table 3, and might be used for pulp mills bleaching with chlorine. A technology-based limit for AOX (tactic no. 1) might be adequate, however, since AOX is a partly-identified mixture of organochlorines, not a specific substance of known toxicity. Other toxic substances (resin acids?) could be specified. However it would seem pointless to use such a chemistry-based approach for pulp mills, with their potentially enormous variety of toxic substances. Instead, the total toxicity would be assessed in the lethal and sublethal tests on the effluent.

End-of-pipe Application of Tactic no. 2

Limits under tactic no. 2 would be decided according to the dilution and mixing at a specific site, but the control point would be just before the end of the effluent pipe, with *calculations* to extrapolate to the edge of the mixing zone. That is the most efficient way to apply tactic no. 2. The limitations for a particular mill would be *measured, applied and enforced* by tests and numerical values for the effluent. Despite the end-of-pipe surveillance, these would remain as water-quality-based limitations, not technology-based ones, because of the calculated derivation from the edge of mixing zone.

Mixing Zones

An arbitrary decision must be made on the allowable size of mixing zone, and some guidelines are available (EPA 1985a, Hanna et al. 1987). A model of physical conditions in the waterbody (currents, size) is also needed. This predictive approach from the discharge point to a mixed condition might be in error, of course, and that is one of the reasons that verification is required by applying tactic no. 3.

Government agencies are often reluctant to designate a mixing zone, because there may be public criticism that part of a body of water has been "written off". Logically, however, there *must* be a mixing zone allowed at each point of discharge, even if it has not been officially declared. If no mixing zone were allowed, that would logically require that the effluent should be of exactly the same quality as the receiving water, i.e. the effluent itself should be suitable for supporting a natural ecosystem, or for swimming, drinking, etc. Some mixing zone, no matter how small, must be allowed either implicitly or by declaration.

A designated mixing zone need not be, and should not be a "zone of death". Indeed such a zone should not have unpleasant conditions, and might not be greatly different from the natural system. The intent should be, however, that only at the outside edge of the zone would the limitations for absolute protection of the ecosystem be imposed to the last decimal place.

TACTIC 3. QUALITY-CONTROL CHECKS BY ECOLOGICAL SURVEYS

The objective of this tactic is to provide a final check on the adequacy of any pollution-control program, for example any predictive errors included in water-quality tactic no. 2. The survey is not a primary control parameter in itself. If significant ecological effect is found, it should feed back to tactic no. 1 and stimulate a tightening of the limits under that technology-based tactic.

This check based on a study of the downstream community water is important, because one can *never* predict effects on a complex system, from observations made on a component of that system. In other words it would be foolish to predict effects on an ecosystem, from a sublethal test with minnow larvae in buckets of effluent.

Ecological surveys are not necessarily large and expensive. The organisms in the receiving water provide economical monitoring of continuing or episodic discharges, integrating and cumulating 24 hours a day and 365 days a year. A small survey can detect change since there is considerable redundancy of information from different groups of organisms, and so the scope of survey can be severely narrowed. For example in freshwater streams, conclusions based only on the mayflies and beetles were essentially the same as from a massive survey of groups ranging from diatoms to fish (Kaesler et al. 1974). Other evidence of ecological effect should not be ignored, but it is not at all necessary to survey all the biota at every survey. Tactic no. 3 may be beneficially based on surveys of bottom-living invertebrate animals. The species differ in sensitivity, identification is feasible, they are fixed to a particular area of the waterbody, and the duration of their life cycles means that surveys are retrospective of damage for previous weeks or months.

Surveys can also be infrequent since they are only checks of the more frequent control measures. An ecological assessment could be done once a year for new or enlarged industrial operations, for a few years, then could be repeated at a 3-year interval.

Criterion of effect

The big problem in using the monitoring tactic (no. 3) is to persuade biologists to specify a criterion or criteria for deleterious effect or "meaningful change" in communities. In particular, the more modern and sophisticated techniques (e.g. cluster analysis, principal components analysis) are aids in interpretation of information, rather than sources of a decision on "effect/no effect". The sophisticated techniques should be used but regulation should be based on simpler criteria (Hellawell 1978, Washington 1984).

A meaningful deleterious effect in aquatic macroinvertebrates might be regarded as a clear and consistent change in any one of the following items:
(a) decrease in number of species;
(b) decrease in diversity index or other recognized biotic index; or
(c) major increase or decrease in number of individuals.

To be considered real, the change would have to be statistically different from whatever was considered to be a baseline or "normal" situation in the body of water at that time. The finding would also have to be clearly the result of the discharged effluent, and not the result of another factor such as change in habitat. There is sampling variation as well as natural positional and seasonal variation in populations, and that would have to be addressed in design of the survey and analysis of results (Green 1979).

Other options for tactic no. 3

Occasionally it is difficult or impossible to use benthic surveys to assess pollution. In some lakes, any effects of an effluent could be confounded with effects of normal deoxygenation of deep water. Sometimes it is very difficult to get valid samples because of rocky bottom or swift current. In such cases artificial substrates may be of assistance (Cairns 1982), other groups of organisms may be assessed, or other approaches relied upon.

Chemical surveys could be included as part of the "ecological" reconnaissance. *Chemistry should definitely be used* if there is potential discharge of a persistent bioaccumulative toxicant. Concentrations of that toxicant should be measured periodically in sediments and/or suitable organisms such as resident predatory animals.

Biochemical/physiological testing of resident fish or other organisms might be added to ecological survey or substitute for some of the surveys. The within-organism variable would require validation, that it was directly associated with meaningful whole-organism effects (growth, reproduction etc.). Some of the most promising biochemical tests for pulp mill wastes would be fish liver metabolites and defence enzymes (especially EROD), extensively studied in Scandinavia (Larsson et al. 1987, Oikari and Kunnamo-Ojala 1987, Andersson et al. 1988, Lindström-Seppa and Oikari 1990, Lehtinen et al. 1990) and now being tried around the world.

A CHOICE AMONG LEVELS OF PROTECTION

If a pollution biologist is asked how much toxic material can be discharged without affecting an aquatic community, the answer should probably be "none", a single option that is difficult to reconcile with present-day industrial operations. As technology changes and as environmental demands shift in light of new knowledge, any single goal for effluent characteristics cannot hope to meet the variety of existing situations, let alone future situations. Therefore it is useful to have a series of "Levels of Achievement" graded for increasing strictness, as options for today and as markers of future progress. The ultimate Level of Achievement might well be an ideal such as "virtual elimination" of toxic substances. Inserting such a final goal into the objectives might help to orient the items in lesser Levels of Achievement, some of which might be changed if they diverted activity away from the final goal.

The approach recognizes the compromises between the biologist's "no discharge" and existing situations for industry, municipalities, agriculture, etc. The concept may be visualized from Table 4, and the range of levels might be set according to the following outline.

Level I would be a base case, probably considered unsatisfactory industrial practice by most people, e.g. a mill that released much waste material and had minimal waste treatment or none. There are now few such pulp mills on the Canadian scene.

Level II would represent a well-run mill with reasonably "low-pollution processes" and primary waste treatment.

Level III is similar to the proposed Federal Regulations as of September 1990 (EC 1990a). Attaining this level of discharge would represent an improvement for most Canadian mills. Some of the items might be achieved by within-mill practice in certain kinds of mills, but secondary treatment would probably be required to satisfy all items.

Level IV might represent a pulp mill with very good control of discharges and secondary waste treatment. This level could be a suitable goal for a decade or more in the future, for example the toxicity limit is considerably stricter than merely non-lethal.

Level V is for a "zero-effluent" mill, allowing for small accidents and leaks. This is in Table 4 as an ultimate objective for industrial research, and cannot be expected for most mills in the near future. The toxicity emission would essentially be equivalent to the volume of discharge.

Table 4. Increasingly stricter levels of achievement for effluent discharge and quality of receiving water. Levels suggested by terms in the left-hand column would be matched by numerical limits in the body of the table.

	CONTROL TACTIC AND ITEMS CONTROLLED		
	(At end of pipe)	(At edge of the mixing zone, and beyond)	
	(1) Technology-based	(2) Water-quality-based	(3) Check by ecological survey
Level of Achievement	Numerical limits for specified variables and toxicity	Numerical limits for specified variables and sublethal toxicity	Limit(s) for degree of change in community.
I (Poor)			
II (Fair)			
III (Good)			
IV (Excellent)			
V (≈ Zero discharge)			

Such a choice of levels may be useful for a number of reasons. First, as mentioned above, it recognizes the present compromises between an ideal and the existing situation. A regulatory agency could select an appropriate level for particular circumstances.

Secondly, the existing quality of effluent at mills could be rated by Level of Achievement, indicating which were most deserving of improvement. Within one mill, different characteristics of the waste may be rated independently for level of achievement, again indicating where the focus should be for improvements. For example there might be excellent control of toxicity at a particular mill, but an unsatisfactory situation for BOD.

Thirdly, economic estimates may be assigned for achieving the different levels, allowing social and economic costs and benefits to be weighed along with environmental considerations.

Finally, the Levels of Achievement could be used to set a schedule for future improvements.

Possible numerical values for the Toxicity Emission Factor are shown in Table 5 under the "Technology-based" column for tactic no. 1. A value of 50 might be suitable for Level IV ("excellent") since that would represent 50 m^3 of non-lethal water (or "just-lethal" water) used for each tonne of product, and in many Canadian mills, current water use would be 80 - 120 m^3/t.

Table 5. Some examples of numerical values that might be suitable for selected items, at the various Levels of Achievement.

	CONTROL TACTIC AND ITEMS CONTROLLED		
	(At end of pipe)	(At edge of the mixing zone, and beyond)	
	(1) Technology-based	(2) Water-quality-based	(3) Check by ecological survey
Level of Achievement	Numerical limits for Toxicity Emission Factor, m^3/t.	Numerical limits for dissolved oxygen, % saturation.	Limit(s) for degree of degradation in aquatic community.
I (Poor)	1000	38	Major decrease
II (Fair)	400	54, summer 60	Measurable decrease
III (Good)	150	70, summer 83	No sig. decrease
IV (Excellent)	50	≈ saturation or no decrease	No sig. decrease
V (≈ Zero discharge)	20	≈ saturation or no decrease	No sig. decrease

Levels of Achievement for Tactics no. 2 and 3

It is more difficult to give a choice of Levels of Achievement for tactic no. 2. The requirements are set by the needs of aquatic organisms which in some respects are absolute, i.e. an ecosystem is either affected or it is not. However that does not necessarily mean a zero contribution of material from the effluent. For example suspended solids are a normal component of waterbodies, and certain levels are tolerated by organisms without apparent damage. Similarly, although any decrease of dissolved oxygen should in theory place a load or stress on aquatic animals, in fact there are variations in natural levels of oxygen and they are tolerated by communities that seem healthy. Similarly, most readers will be familiar with the concept of no-observed-effect concentrations for the usual toxic substances.

Limits may thus be recommended which are not the same as pristine conditions, but might allow ecosystems to function at apparently normal levels of vigour, production, and diversity. Limits may be chosen from accepted water quality criteria and from assessments of sublethal toxicity. In some cases a series of Levels of Achievement/Protection may be assigned in a reasonable manner and occasionally they have already been provided, as for dissolved oxygen.

The suggested degrees of saturation of dissolved oxygen given under the water quality column of Table 5 are taken with a slight amount of simplification from the recommendations of Davis (1975) for mixed populations of freshwater fish including salmonids.

> **Level of Achievement I for oxygen**, in Table 5, represents "Protection Level C" of Davis, who provides a description. "... a large portion of a ... fish community may be affected by low oxygen. This deleterious effect may be severe, especially if the oxygen minimum is prolonged beyond a very few hours."
>
> **Level of Achievement II** adheres closely to Protection Level B of Davis. "... the average member of a ... fish community starts to exhibit symptoms of oxygen distress."
>
> **Level of Achievement III** is taken from Protection Level A of Davis. "... few members of a ... fish community, will likely exhibit effects of low oxygen at or above this level."
>
> **Levels of Achievement IV and V** are not taken from the recommendations of Davis (1975), but simply represent a requirement for no degradation, recognizing the principle of theoretical stress from any decrease in availability of oxygen.

Similarly it is difficult to set degrees of allowable change in an ecological survey (the last column of Table 5, the quality check under tactic no. 3). The verbal descriptions for Achievement Levels I and II would have to be replaced by quantitative or numerical criteria. Levels III to V are shown with the same description; a "good" status would seem to indicate no measurable deleterious effect on a community, and so "excellent" could not be demonstrably better.

COMPARING SOME NATIONAL REGULATORY STRATEGIES

Australia

In December 1989, Australia produced a package of guidelines for discharges from bleached kraft mills, that is possibly the world's neatest, most comprehensive, rational and brief. It is also very strict (Australia 1989). To some extent the program involves excessive testing, but there are provisions for reduced frequency, given favourable results. The package was the product of a deliberate and remarkably speedy program of study, following an absence of standards in early 1989 which resulted in the cancellation of a planned mill.

The guidelines included all three tactics recommended here, and most of the Australian items concerned with water quality are reorganized in Table 6, along with any specific limits, and frequency of testing in parentheses.

Table 6. Australian guidelines for regulations, released in December 1989, and summarized here under the three recommended tactics. Cl'd signifies Chlorinated.

(1) Technology-based limits:		
TSS	8 kg/ADt, 24-h composite.	(daily or weekly)
BOD	7 kg/ADt, 1-d maximum.	(weekly)
Oil and grease	No visible contamination.	(daily)
AOX	2.5 kg/t maximum, 1 kg/t yearly moving average.	(weekly)
Cl'd dioxins/furans and Cl'd phenolics, chloroform	No increase over background.	(monthly)
Acute lethality	LC50 ≥100% effluent for daphnia and trout, at minimum water use of 50 m^3/t. Probably also Microtox.	(monthly)
(2) Water-quality-based limits, edge of mixing zone of diameter 500 m:		
Effluent	Dilution ≥200 to 1.	
Sublethality	<NOEC for fish and invertebrate (tested with effluent, 3-monthly)	
Colour	<20 units at surface.	
In water	TSS, DO, chlorate, AOX	(3-monthly)
In water	Cl'd phenolics	(yearly)
In sediments	EOX, Cl'd phenolics, Cl'd dioxins/furans, and sublethal toxicity test with a burrowing organism.	(yearly)
(3) Checking, monitoring:		
EOX, Cl'd phenols	Measured in biota.	(yearly, 3-monthly)
2,3,7,8 TCDD	No detectable increase in water.	
TCDD equivalents	Limit 5 ppt, crustacean hepatopancreas, mixing zone edge.	(yearly)
Communities	Benthic populations, outside mixing zone.	(3-monthly)
Fish	Fish abnormalities, histopathology, liver MFO, seafood tainting, outside mixing zone.	(3-monthly, or yearly)

For any chronic impact found under the Australian scheme, the cause is to be discovered, and the regulatory authority would determine action to be taken. After 3 years, then at 5-yr intervals, there would be a review of the monitoring program, effects on aquatic ecosystems to ≥10 km, and an assessment of testing, possibly leading to adjustment in programs.

United States of America

The U.S. now has a good national regulatory approach for effluents, encompassing all three tactics recommended above. Federal discharge permits are normally handled by individual states, but U.S. EPA retains a veto power and acts if not satisfied with state standards. Many states now use a complete spectrum of regulation, based on: (a) best technology; (b) lethal and sublethal testing; (c) site-specific water quality criteria for individual chemicals; and to some extent (d) receiving-water surveys. Several U.S. speakers at this workshop have described aspects of the program.

For conventional pollutants, EPA set limits of TSS and BOD for 33 categories of processes in the pulp and paper industry, but essentially requires the equivalent of secondary waste treatment (BAT). Effectively all U.S. mills now have secondary treatment, and output of conventional pollutants is generally lower than requirements. Examples (Bonsor et al. 1988) of limits in terms of output per tonne of product are:

	TSS, kg/t	BOD, kg/t
Bleached kraft pulp	9.5	3.8
Semi-mechanical pulp	5.5	2.1

For toxic substances, the dominant U.S. approach from the late 1960s to the early 1980s was water quality criteria and standards for individual chemicals (part of tactic no. 2), and these continue to be used. Numerical estimates were made of "safe" concentrations of various toxic chemicals, and the famous list of 129 "priority pollutants" was applied to industries as the substances were thought to be relevant.

Toxicity testing was not part of effluent *regulations* in U.S.A. throughout the era of water quality criteria, although some testing was done. The main administrators of EPA were apparently unaware of effluent toxicity tests in other countries, but toxicity requirements were suddenly adopted with enthusiasm in the mid-1980s. A very advanced and rational use of lethal and sublethal testing in discharge permits was developed, and might well be emulated in other countries (EPA 1984, 1985a, 1985b; Giattina and Anderson-Carnahan 1986). Test programs vary with the state, but EPA recommends the following.

Tests of acute lethality, fitting tactic no. 1 of the present paper.

> Tests on a fish, an invertebrate, and a plant, often the fathead minnow, the waterflea *Ceriodaphnia* sp., and an alga. The most sensitive organism during the first two months of testing is used for later tests. The lethality tests are used in one of three ways in various regions or states, but generally, peak concentrations must not be lethal outside a small initial mixing zone, and sometimes full-strength effluent must be non-lethal.

Sublethal tests, a site-specific requirement fitting tactic no. 2.

> Three species are tested, ordinarily: (a) 7-day growth and survival test with newly-hatched fathead minnows; (b) 7-day *Ceriodaphnia* test for survival and number of young; and (c) growth during 4 days of an alga (*Selenastrum capricornutum*). The NOEC must be higher than the average concentration expected at the edge of a mixing zone. In a river, the specified dilution is low flow, normally the 10-year minimum for 7-day average flow (the 7Q10).

Lethal and sublethal tests might be required monthly for the first year and twice a year thereafter. Calculations to the edge of the mixing zone are made as toxic units (lethal or sublethal) to simplify the arithmetic. The limit based on acute lethality is intended as a maximum one-hour peak but in practice is a maximum for the daily average. The limit based on NOEC is a maximum 4-day average (EPA 1985a).

Finally, under tactic no. 3, U.S. permits often require one or both of biological or chemical surveys in the receiving water, similar to the Australian guidelines. The entire U.S. approach is complex and still has some blind spots, but the combination of items provides comprehensive environmental protection. In particular, toxicity tests with three types of organisms are desirable, to include any major differences in sensitivity between general groups.

Canadian Federal Government

The Canadian government promulgated regulations and guidelines limiting discharges from pulp mills almost two decades ago, and they were good for their time (EPS 1971/72). For example they limited unbleached kraft mill discharges of TSS to 10 kg/tonne of pulp and BOD to 16.5 kg/t (i.e. tactic no. 1).

There was a requirement for non-lethality to trout, concentration-based and therefore subject to distortion by the mill's water use, but still a pacesetting move by Canada in use of biological testing. I can recall Terry Howard of B.C. Research saying at an Aquatic Toxicity Workshop in Burlington, that "no other country demands that industry satisfies a biological criterion of acceptable effluent quality", and that the tests had often been the only factor stimulating the choice of less-toxic processes and secondary waste treatment in the pulp and paper industry (Howard 1978).

Compliance with the 1971/72 limits did not turn out to be outstanding, however, for example a decade later only 36 of 123 mills were known to pass the fish toxicity test (EPS 1984). Recent data indicate that most mills are well below the limit for TSS, but almost half of them exceed 16.5 kg BOD/t of pulp, and most still do not meet the toxicity requirement. Many escaped the regulations, which did not apply to existing unmodified mills.

Since issuing those regulations, Environment Canada has moved at a snail's pace, and senior bureaucrats seem caught in a time-warp of concepts from 1971. New regulations are in development, but the "new" version being discussed at the time of writing, indicates only a stricter and more highly polished version of the old limits described above. The proposed numerical limits may be based on the quality of effluent from good secondary treatment (TSS 11 kg/t ?, BOD 7.5 kg/t ?, fish LC50 >100% ?). The limits are not particularly strict.

In addition, limits on chlorinated dioxins and furans will be issued under the Canadian Environmental Protection Act. Limitation of general organochlorines (AOX) has been under consideration by Environment Canada for almost two years at the time of writing, and probably will not be developed.

All of the proposed new Canadian federal regulations continue to fall under tactic no. 1, the "good practice" or BAT option. Managerial eyes seem to remain steadfastly fixed on the end of an effluent pipe, seemingly unaware of an ecosystem approach.

Federal movements toward site-specific water quality limits (tactic no. 2) are poorly defined. An impressive book of *Canadian water quality guidelines* exists (CCREM 1987), but there are no legislated requirements to meet those guidelines, and no use of them in the proposed pulp mill regulations. Canada Dept of Fisheries and Oceans may assist in injecting tactic no. 3 into the new regulations, as some form of periodic environmental monitoring.

Control Activity by Canadian Provinces

The ten provinces usually take an integrated, case-by-case approach, with pollution control as an empirical blend of tactics no. 1 and 2. Pollution surveys (tactic no. 3) are done by many provinces, sometimes very sophisticated ones (e.g. Anderson 1989). The major pulp-producing provinces also proclaimed objectives for AOX in kraft mill effluents in April-June 1989, or else have ensured that new mills would have state-of-the-art processes with low discharge of AOX.

There are also discouraging signs among provincial agencies. Ontario deliberately abandoned its excellent base of water quality objectives under a developing control program ("MISA", for Municipal-Industrial Strategy for Abatement). Ontario's efforts have been diverted to a massive program of measurements, chiefly chemical, intended to produce controls based on BATEA.

GODZILLA ON A RAMPAGE

There is an unfortunate contrast between on one hand, the call to think at the biosphere level, protect ecosystems and change rapidly, and on the other hand a creaking process which appears to be grinding out regressive regulations for control of water pollution. The regulations are examined below for their conformance with a number of Suzuki-style items.

Thinking biosphere

Still using the example of pulp mills, the principle is that specific regulations for effluent control should be framed within the broadest possible considerations of environmental benefits and debits. A particular conclusion is that **obligatory waste treatment is not necessarily desirable.**

There is a strong and natural tendency to set regulatory limits at the levels achieved by good secondary treatment. The proposed federal regulations for pulp mills are clearly intended to force the mills to install secondary treatment. There is no particular environmental logic in that and it is conceivable that full treatment might not be the best choice for the biosphere, in some situations. The following aspects should be considered.

If secondary waste treatment is installed, environmental attention becomes focused on that operation. A mind-set is developed, not to be concerned with how much waste is generated because "it will be treated anyway". Short-term regulations that focus activity on operation of waste treatment facilities will almost certainly divert attention from what should be the long-term goal of the no-discharge mill and recycling of materials within an industrial operation. It is much better to avoid creating a waste material, than to produce it and then expend energy and resources to make it disappear. A good example of this principle is the steps taken in the last few years by kraft mills around the world, to change bleaching processes and thus reduce production of waste organochlorines.

Talk of a no-discharge mill is not naive. Strong environmental pressures are driving mills towards no discharge, or much reduced discharge, in many parts of the world.

- In mid-1989 a proposed kraft mill in Australia was abandoned after much expense, for fear of overly-strict environmental regulations (Australia 1989).
- In Alberta, environmental hearings in late 1989 stopped a proposal for the world's largest kraft mill, pending further information on potential pollution (ALPAC Review Board 1990).
- A bleached CTMP mill planned for Saskatchewan proposed that two years after start-up, it would have no effluent. The provincial government response went beyond even that, however, and requested no discharge from the beginning, with a consumptive water use of only 3 m^3/tonne (Noble 1990).
- The International Joint Commission recommends that Lake Superior, with half-a-dozen pulp mills discharging on the Canadian side, be designated "as a demonstration area where no point source discharge of any persistent toxic substance will be permitted" (IJC 1990).

At some locations, waste treatment could have net detrimental aspects from a "global" environmental point of view, since it is not without inputs of energy, materials, land, and problems of sludge disposal. There is no hint that Environment Canada factored these other things into the equation when framing regulations.

Energy use should be considered. BOD in an effluent can be reduced, but what about the giant 50 HP aerators in a treatment pond? Perhaps there is only a small production of CO_2 resulting from the generation of electricity for those aerators, but perhaps in certain locations it might be better to let natural processes bring about oxygen equilibration in the receiving water. If the effluent is relatively benign with respect to persistent toxicants, why decompose some waste components of wood in a man-made, energy-intensive pond on the land, if nature will do the same thing quietly, effectively and efficiently? The CO_2 from decomposition would still be produced, but there would be a saving on the 50 HP aerators, since aeration of a waterbody would be provided by existing wind-power. If that seems shocking, it can be enlightening to calculate BOD inputs to a big lake from a pulp mill, compared to natural inflows.

Obligatory treatment may waste economic resources that would be better employed in remedies for other environmental problems. The proposed regulations represent a philosophy of "one size fits all". They cannot avoid being either over-restrictive or under-restrictive for any given receiving water, whether the Atlantic Ocean or Trickle Creek, Saskatchewan. Economists apparently have many economic models predicting that such a regulatory process is **guaranteed to give the smallest bang for a buck**. A number of studies indicate that instead of using "command and control" regulations like those proposed, incentive-based regulations can achieve the same results at costs that are lower by factors as great as 15 (N.C. Bonsor, Lakehead University, personal communication).

Mass loading of toxicity

Acute lethality is included in the proposed regulation for effluent monitoring under technology-based tactic no. 1. However there is no official recognition of the concept that the **amount** of toxicity entering an ecosystem is the important thing. The concept has been championed by various younger or more scientifically-minded public servants (e.g. Wilson et al. 1975) but it has never emerged in regulations proposed by Environment Canada. The pulp and paper industry has shouted the message without apparent effect. An example was contained in a submission on the proposed regulations from a category of mills that may have a very small effluent that is toxic (CTMP mills -- Chemi-Thermo-Mechanical Pulp, and TMP and other combinations). As shown in Table 7, the common kind of kraft mill could discharge seven times as much toxicity as a TMP mill, without failing the regulatory test.

Table 7. Example of different quantities of toxicity that might be discharged from two different kinds of pulp mills, both meeting the proposed federal regulation for fish toxicity. From a presentation by the CTMP industry to Environment Canada workshop, June 1, 1990, Ottawa.

	TMP Mill	Modern Kraft Mill
Toxicity, LC50	100 %	100 %
Production, tonnes/day	950	950
Water consumption m^3/t	17	120

The kraft mill discharges seven times as much toxicity.

Thus the regulation fails to encourage water conservation, and discharging a carload of effluent is apparently given equal weight to discharging a pailful. The message to the mills is clear -- if there is a problem in meeting the lethality requirement then use as much water as possible in the effluent to dilute the toxicant. The regulation allows cooling water to be combined with process streams.

Fish lethality test outmoded

The lethality test with fish, which Canada pioneered for effluent regulations, should no longer be given primary place in assessing toxicity. Sublethal effects are more important to assess in the final analysis, and we are now able to measure them in tests as short as 4 days.

It is becoming more difficult to get excited about whether an effluent is lethal at the end of a pipe -- it may or may not mean anything important. It may mean that resin acids are present, but they will decompose in 6 weeks in the receiving water and are not the persistent toxicants of most concern nowadays. Death in a lethal test may mean only that the pH was 11.5 and killed the fish -- it is an archaic incongruity that one is not allowed to adjust the pH in the proposed regulatory lethal test. And yet pH will be almost instantaneously buffered when the effluent is discharged into the harder types of surface waters (exception: soft-water lakes, already stressed). The toxic effects of pH are well documented and if Environment Canada is sincerely interested in measuring those effects within an effluent pipe it would be cheaper to use a pH meter than a fish toxicity test.

If one is concerned about subtle persistent bioaccumulative toxicants such as chlorinated organics that came from some kinds of pulp mills, the fish lethality test probably does not measure them. By focusing exclusively on lethality as a measure of toxicity, Environment Canada is in fact diverting attention from the important issues of toxicity.

This misleading aspect of a lethality test can direct activity down paths that may not be correct from the overall biospheric viewpoint. I have visited mills which had decided to install activated sludge as a treatment method, after considering different alternatives such as lagoons, or anaerobic systems which would reduce BOD and also produce methane gas for useful energy recovery. When asked the reason for choosing activated sludge which has (a) high capital cost, (b) high operating cost, (c) high energy input with no energy recovery, and (d) little better control of the traditional pollutants, the reply was that "pilot-plant tests have shown that it is more likely to eliminate toxicity", by which they meant acute lethality to fish. Relative effectiveness on subtle persistent toxicants had not been considered.

Water quality concepts

It is puzzling that organizations outside the federal government can recognize the need for using water quality objectives in regulations, but Environment Canada does not do it. The outside recognition was indicated above by the quotation from the Canadian Wildlife Federation at the start of the section describing tactic no. 2. The deficiency is all the more puzzling because the existing Canadian water quality guidelines (CCREM 1987) are, as mentioned above, excellent. Those existing numerical criteria have not been used for regulations under tactic no. 2, nor in the proposed methods for monitoring environmental effects of pulp mills (see following section).

The concept of checking by ecological monitoring

The ecosystem concept elicited some recognition in the package of proposed pulp and paper regulations, but a draft document intended to give guidance on "Environmental Effects Monitoring" (EEM) was an example of hopeless confusion (EC 1990b). The concept of field assessment of communities to check on pollution control (i.e. tactic 3) was lost in alarmingly long lists of recommended tests that would more properly belong under tactics no. 2, 1, or zero.

The draft EEM document had two major faults which doomed it to uselessness. First there was no clear recognition of the major purpose or objective of "effects monitoring", namely checking wild communities of organisms to see whether or not there is an effect of the effluent. Failing that recognition, the document instead provided the mixed lists of tests from the three tactics.

The second deficiency was that there were absolutely no indications for any of the tests, of what would be an acceptable finding and what would be unacceptable (concentration in the water, degree of effect shown by an organism, or whatever). Without that there is no sign that any of the tests is a useful thing to do. Some of them are clearly not useful at present because we do not know what the results mean, and cannot use them to detect a meaningful effect.

As it stood in June 1990, the document required many tests, for example the following requirements would be mandatory for a bleached kraft mill. The expense would be considerable, perhaps $200 or 300 thousand to run through the list, and most of the items would apparently apply to even small specialty paper mills for which the cost would be ruinous.

- 24 physico-chemical tests on the effluent, 8 of them various resin acids and some others being groups of chemicals (e.g. chlorophenols) requiring individual breakdown.
- Lethal tests with the effluent on trout and *Daphnia*, and sublethal tests on fathead minnows and *Ceriodaphnia*.

(Those are mostly tests to assess the quality of an effluent, and do not monitor effects in the receiving water. The sublethal tests could be used in a predictive manner for tactic no. 2, if such a purpose were stated and a procedure for doing it were defined in the document.)

- 14 physico-chemical tests in the receiving water and 4 in the sediment (of which perhaps 5 are important enough and well enough understood that they might be used for effects-monitoring, if allowable values were stated in the document).
- Sublethal tests on the receiving water with fathead minnows and *Ceriodaphnia*. (These might or might not detect important effects, depending on when samples happened to be taken.)
- Acute lethality test with sediment (a potentially useful monitoring device if sublethal testing had been specified instead, since the sediments can act to cumulate or integrate levels of pollution over time).
- Tainting and dioxin/furan residues in fish from the receiving water, useful and important tools under monitoring tactic no. 3. However, 4 additional required measurements of residues and biochemistry are of uncertain usefulness at this time.
- Surveys in the receiving waterbody of benthic macroinvertebrates (excellent), but also of fish (expensive and poor indicators) and algae (life cycle too rapid to be good indicators).

Thus the draft EEM document did manage to list useful items for checking effects, the proper purpose for "effects monitorint", but failed to single them out as important amongst its scattergun list of dozens of items. Additional tests were listed and might be required of a mill by Environment Canada. The whole approach is the opposite of good science, the essence of which is to design the simplest possible experiment to answer the question at hand. The document appears to design the most complicated possible testing program, in order to answer questions that have not been defined. One might attempt to remedy the draft document by removing things. All tests should be removed that do not measure effects on wild communities of organisms in the receiving water. That would fit the presumed purpose of environmental effects monitoring, to see if there actually was an effect on the receiving communities of organisms. Certain other categories of test might be added back for specific purposes, e.g. wild or caged individual organisms for biochemistry or tainting. All tests should be removed that were not accompanied by a numerical statement of what would be favourable and unfavourable results. That would eliminate expensive busy-work and focus efforts on meaningful tests.

The final absurdity of the draft EEM document is that Environment Canada apparently has not considered who is going to receive and catalogue all the information generated, nor decided how it will be processed and stored, let alone who will evaluate it and detect any unacceptable results. Anyone who has handled test-data and perhaps tried to store it on a computer base, might well feel daunted at the prospect of a flow of results from at least 54 kinds of tests and surveys, from 120-odd Canadian pulp mills, with some of the information at intervals of a month or perhaps more frequent.

It appears that Godzilla is alive and well in Ottawa. It does not appear that Dr. Suzuki's message has collected a strong following in that place.

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PLENARY SESSION

Chair: D. Johns

17th ANNUAL AQUATIC TOXICITY WORKSHOP

Presentation by
Mr. Cliff Serwa, MLA
Parliamentary Secretary to the
Minister of Environment for British Columbia

TITLE:

"WATER QUALITY: WHAT THE PUBLIC NEEDS...WHAT THE PUBLIC WANTS"

SUMMARY:

Public concern about water quality has grown dramatically due to media coverage and mounting evidence of toxic impacts. Community residents expect public agencies to provide clear, factual information on impacts and risks, and to ensure that corrective action is taken as quickly as possible.

TOXICOLOGY IN ENVIRONMENTAL DECISION-MAKING

By

Earl D. Anthony

INTRODUCTION

It is my pleasure to welcome you to Vancouver, and to the 17th Annual Aquatic Toxicity Workshop. In looking over the agenda, I see 160 speakers who will be heard by over 400 participants. The growth in size and scope of this annual meeting is perhaps a sign of the growing importance of toxicology in environmental decision-making. The last time this workshop was held in Vancouver, in 1984, 123 participants heard just 60 presentations.

I would like to tell you about the trends in biotesting in Environment Canada, and how toxicology is being used in environmental decision-making in the Pacific and Yukon Region. My talk will mention the traditional use of bioassays in monitoring and enforcement, and outline the expanding role of toxicology in the regulatory framework. Finally, I will give some idea of where I see us going in the next few years.

THE 96 HOUR FISH BIOASSAY

When Environment Canada was established in 1971, the only bioassay in wide enough use to be accepted by the courts was the 96 hour rainbow trout bioassay. It was established as legal precedent early on that this bioassay shows whether a substance was or was not "deleterious" within the meaning of the Fisheries Act. Coho salmon underyearlings were used until 1975, when rainbow trout became the standard test species. By 1980 the federal procedure was standardized, followed by the provincial procedure in 1982.

This test provided a level playing field for companies and municipalities discharging effluents to a wide variety of environments. No matter whether you ran a mine, a pulp mill or a sewage treatment plant; no matter whether you discharged to a little creek or the Pacific Ocean, failing a 96 hour fish bioassay meant trouble, and still does.

We have long recognized that lab testing sometimes bears little relevance to toxicity or other deleterious effects in the real environment; but we had a standard test that could be required as part of a provincial waste management permit, or required under federal regulations. Everybody knew what it meant. Government and private labs could do it, and get similar results with the same effluent.

Since 1971 the lab has averaged approximately 400 bioassays/year, of which an average of 12% are legal (i.e., supporting enforcement actions and may be used as evidence in court). Figure 1 shows the number of legal and other bioassays performed in our lab from 1975 to present. During the past year our joint federal-provincial lab bioassayed 467 samples using rainbow trout. Just under half of these were for provincial and other clients (Figure 2). Of the total, 135 were legal samples.

BIOTESTING IN THE NEW REGULATORY FRAMEWORK

By the mid-1980, a new regulatory regime was emerging, driven primarily by three factors:

-technology had improved, so that regulations based on technology in the 1970's were obsolete;

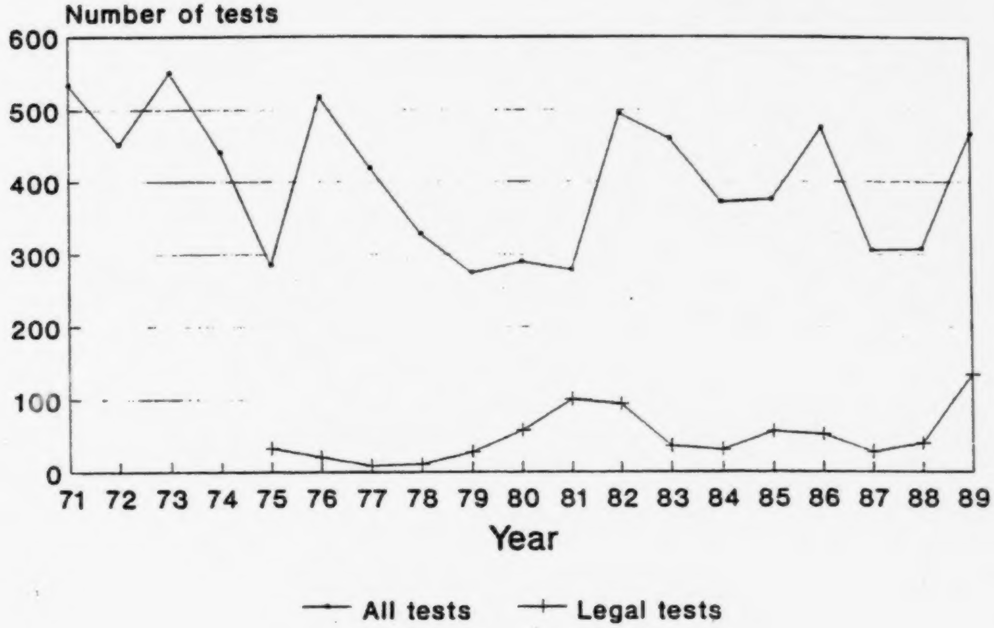
-the toxicology data base and methods had improved, so that real effects on the environment could be more confidently predicted and assessed;

-the public was demanding better environmental protection.

By 1986 Environment Canada offered 23 kinds of bioassays in addition to the 96 hr. rainbow trout bioassay (Sergy, 1987). Included were lethal, sublethal and bioaccumulation tests for a variety of fish, invertebrates, algae, protozoans and bacteria. A need to develop standard protocols for a core of nine tests for regulatory programs was recognized. Both freshwater and marine species are included. The Intergovernmental Aquatic Toxicity Group (IGATG) began drafting these protocols, and research on others is continuing. Our bioassay lab, which, by the way, shares space and workload with provincial toxicologists, has done much of the developmental work. We now offer a wider variety of tests, including Microtox, Echinoderm larvae, Oyster larvae, clam reburial and contaminant uptake, and amphipod sediment bioassays. These tests are being used regularly by our Marine and Freshwater biology groups to assess impact of pollution. Several of the papers to be presented in the two Toxicology and Regulations sessions today and tomorrow will elaborate on this work.

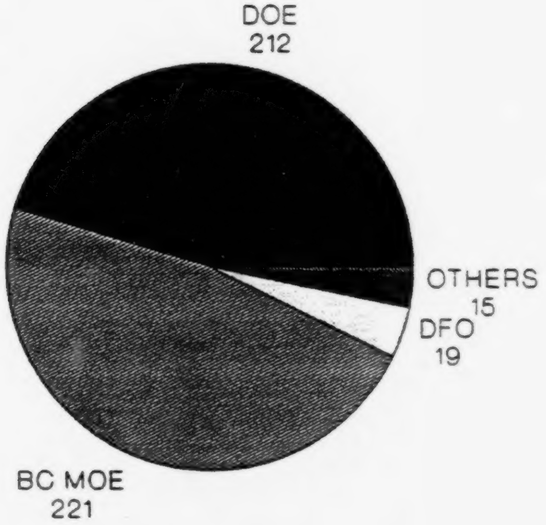
Meanwhile, the Canadian Environmental Protection Act (CEPA), was passed 1988, specifically for the control of toxic substances (among other uses). It mandated a greatly expanded role for toxicology. Among its provisions:

FIGURE 1. ENVIRONMENT CANADA BIOASSAY LAB 96 HOUR ACUTE FISH BIOASSAYS



Coho until 1975; then rainbow trout

FIGURE 2. ENVIRONMENT CANADA BIOASSAY LABORTORY COOPERATIVE SERVICES



-Part I **requires** that the Minister of the Environment formulate environmental quality objectives and guidelines, implying a need for toxicological data;

-A new, broad definition of toxicity was established in Part II,

-Certain substances were established as "toxic" *a priori*

-A Priority Substance List (PSL) of 44 compounds or classes of compounds was established. These must be assessed to determine whether they are "toxic" within the meaning of CEPA, also within a specific time period. One assessment, for dioxin, has been completed. For substances found to be toxic within the meaning of the Act, regulations are anticipated.

-Part VI (previously the Ocean Dumping Control Act) prohibits ocean dumping except as prescribed. It also lists factors, including toxicity, to be considered in granting permits.

While this Act was in development, lawmakers and regulators met to consider the place of biology in the new, regulatory framework (Day et al, 1988). They made seven key recommendations:

1. The Federal government develop a national framework for biological toxicity tests and make the policy decisions required to set biology-based regulatory standards in place.
2. Biological testing and monitoring must be integrated with chemistry in hazard assessment and regulatory control.
3. Sublethal and chronic tests need to be developed to provide more sensitive monitoring tools for detecting toxic effects in ambient waters.
4. Improve communication, awareness and understanding of the applied uses of biological toxicity tests amongst the scientific community, regulatory agencies, industry and the public sector.
5. Maintain a strong research and development capability.
6. The Federal government should provide guidance and show leadership in the area of biological testing and monitoring.
7. Government should be responsible for long-term, ambient monitoring while industry should be responsible for assessment of immediate areas of impact, with occasional auditing by government.

Since then these recommendations have been driving our biotesting programs. I'll give you some examples.

VANCOUVER HARBOUR

During 1985 to 1987 our Marine Biological Group studied environmental conditions in Vancouver Harbour (Goyette et al 1987; Goyette and Boyd, 1989). They found that some Harbour sediments are contaminated with Polycyclic aromatic hydrocarbons (PAH), and that up to 75% of English sole in contaminated areas had preneoplastic or neoplastic liver lesions. Results of continuing studies will be presented in the histology session on Tuesday. PAH is known to cause these kinds of lesions. A multi-agency Action Plan for cleanup and prevention of further pollution has been prepared. A key element is to assess the impact of contaminated sediments, and that is where toxicology comes in.

Partly by contract, and in cooperation with DFO (Institute of Ocean Sciences) we tested sediments from 20 stations in the Harbour with a suite of five different bioassays. Infaunal communities were inventoried at each stations. The toxicological and chemical results integrated with chemical data. This method of integrating chemical, toxicological and ecological data, the "Sediment Quality Triad," will be presented in the Sediment Assays session on Wednesday.

PULP AND PAPER EFFLUENT REGULATIONS

The first federal regulations to reflect the new biological testing framework will be the revised Pulp and Paper Effluent Regulations (PPER) of the Fisheries Act. Passed in 1971, they currently contain a toxicity provision based on a 96 hr. rainbow trout bioassay (an exceedingly expensive, cumbersome, flow-through test). Revisions to these regulations have passed the public consultation phase and will hopefully be promulgated during 1991. The standard (96 hr. static) trout bioassay will be retained as a first-level control; however, a mandatory environmental effects monitoring program (Environment Canada, 1990) has been added. Its purpose is, among other objectives, to determine the adequacy of existing control requirements and the need for more stringent or site-specific measures. Its core program will include chronic sublethal and lethal toxicity tests using fish, invertebrates and algal and genotoxicity. Effluents, receiving water and sediments are included as the test media.

PESTICIDE TESTING

It is surprising that, 30 years after Rachel Carson published Silent Spring, there is still a lack of basic knowledge of the toxicity of many pesticides. Faced with the frequent need to advise provincial authorities on pesticide permits for sensitive areas, our Contaminants group began a pesticide testing program in conjunction with the bioassay lab. They use multiple species tests in a water/material matrix, and many of their findings have been published in scientific journals. Our advice now is backed up by solid data, which meets the secondary need of expanding the toxicological literature.

OCEAN DUMPING

CEPA Part VI (previously the Ocean Dumping Control Act) bears special mention, because of the recent high profile of some our decisions. As I noted earlier, the Act gives toxicity as one of the factors to be considered in approving applications. We began testing various sediment bioassays to assist in these regulatory decisions in 1985 (Envirochem Services Ltd., 1985).

The need for such tests took on added urgency with the release of our reports on Vancouver Harbour (Goyette et al 1987; Goyette and Boyd, 1989).

Busy seaports need continual maintenance dredging. This applies not only to Vancouver harbour, but to other harbours and industrial wharves. How could we continue to approve ocean disposal applications for the dredge spoils, knowing of PAH contamination and the effect on sole and presumably other marine life?

By 1988 we developed a tiered testing approach (E.V.S. Consultants Ltd., 1988) to regulate ocean disposal of dredge spoils: if dredge spoils failed chemical tests (i.e., PAH above a screening limit), the applicant could test it with two sediment bioassays. One of these (the amphipod survival bioassay using *Rhepoxynius abronius*) tests for acute toxicity, while the other tests for developmental abnormalities (using either sea urchin or oyster, *Crassostrea gigas*, larvae). The PAH guidelines were developed in 1989, and the procedure became operational this year. We have since had ocean dumping applicants run the bioassays only 10 times. Five of these were forest industry related and five were general product docks or marinas.

Unfortunately, the results have been somewhat equivocal. Even in dredge spoils with PAH levels well above the screening limits, amphipods survived. But all the larval bioassays failed, including the controls. Obviously, we need more experience in selecting and applying these tests and in interpreting the results. The points out the need for continued research.

RESEARCH

The source of funds for much of the bioassay developmental work I've just mentioned was the Ocean Dumping Research Fund. Since 1976 it funded or provided seen money for 75 research projects in this region. Ten of these, or 14%, related to sediment toxicity or histology (Figure 3). That fund is now gone. It is absolutely essential to seek new sources of funding to provide the scientific foundation necessary for good environmental management.

It is my intention to build partnerships with the universities, and with the federal and provincial granting councils, to further research that gives us new knowledge of fate and effects of toxic chemicals. For example, UBC has long cooperated with the Canadian Wildlife Service on wildlife toxicology issues, such as effects of maternal dioxin contamination on heron chicks. More recently, NSERC has become a partner with Environment Canada in a Chair for wildlife toxicology recently established at SFU.

But we also need more mundane toxicology that does not advance the frontiers of science. We need a stable of routine tests, the workhorses, that build up the database of effects of different mixtures of contaminants at different concentrations under different conditions of the natural environment. We also need a cadre of experienced practitioners to apply and interpret these tests in the regulatory context: to answer the question, "So what if it's contaminated?".

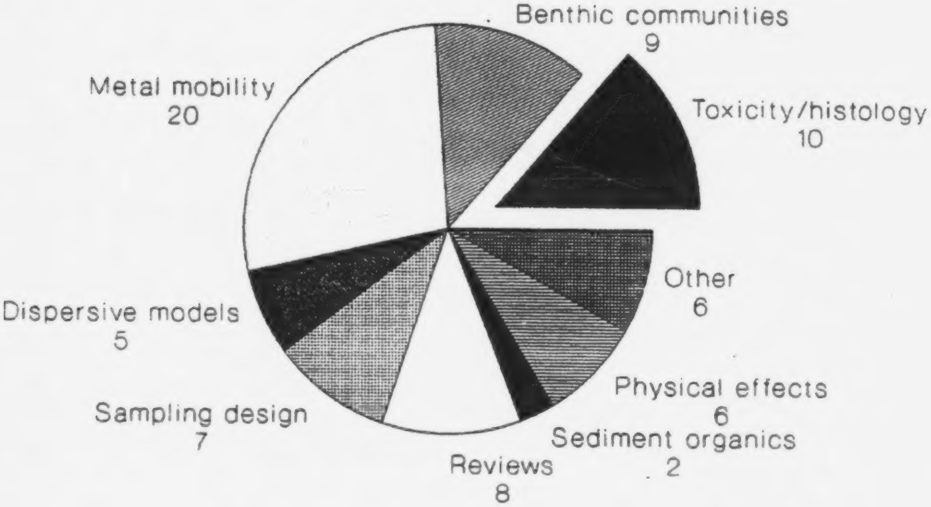
CONCLUSION

I have mentioned something of our history in using bioassays: from the standard, 96 hour rainbow trout acute toxicity bioassay to newer ones whose endpoints are a variety of sublethal effects. I have outlined the expanding use of biotesting in the areas of environmental effects monitoring, pesticide testing, contaminated sediment assessment and ocean dumping. I have indicated some new directions for research in toxicology.

On the far horizon (of the planning cycle) I see environmental managers who:

- are much more confident in their ability to select and apply bioassay tests;
- have greater knowledge of what levels of contaminants do what harm to what species;
- have a greater awareness of relationships between the living components of the ecosystem and their physical and chemical environments;
- can use the principles of toxicology and ecology

FIGURE 3. OCEAN DUMPING RESEARCH BY CATEGORY, 1976-1989



to make sure that the wastes we authorize for discharge are fully compatible with conservation and protection of the marine environment.

And I'm confident that the programs we're putting in place now, with the help of toxicologists such as yourselves, will make this vision a reality.

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BIOLOGICAL EFFECTS OF CADMIUM ON A SMALL PRECAMBRIAN SHIELD LAKE: IS THE CANADIAN WATER QUALITY GUIDELINE SAFE?

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INTRODUCTION

Research at the Experimental Lakes Area, Freshwater Institute, on pollutants subject to long-range atmospheric transport (LRTAP), began by focussing on acids. Because of the large contributions of acidic precursors by nonferrous metal smelters in Canada, it was evident that LRTAP pollutants must include metals as well as acids. Cadmium is a relatively volatile metal that is distributed by LRTAP. It was chosen in the late 1970's as the metal for study principally because it is highly toxic to aquatic life and is found in some freshwaters near to or exceeding toxic concentrations. Globally, six times as much cadmium enters water from anthropogenic sources as from natural sources. Finally, unlike for Hg, regulations set to protect human health in respect to Cd (e.g. 5-10 µg/L for drinking water) are too high to protect aquatic life. Various jurisdictions have guidelines or objectives for protecting aquatic life from Cd, many of these objectives vary with the hardness of the water. The Canadian Water Quality Guideline for Cd is 0.2 µg/L for water of hardness less than 60 mg/L as calcium carbonate.

WHOLE LAKE EXPERIMENTAL APPROACH

As for other contaminants, the effects of Cd were studied at the Experimental Lakes Area (ELA), northwestern Ontario by experimentally adding it to a whole lake. The main objective of this experiment was to describe the geochemical and biological pathways of low levels of Cd from between water, sediments and organisms and to determine the progression of stress caused by Cd from the molecular to the ecological levels of organization. The experiment permits us to test the adequacy of the Canadian Water Quality Guideline of 0.2 µg/L in protecting the biota of soft waters. It also permits us to observe the importance of the sediments as a sink for Cd; to identify some responses which may serve as early-warning indicators of impending ecological damage and to assess the predictive value of results from small-scale, laboratory, microcosm or mesocosm experiments against the response of individuals and populations exposed in the lake. At least 30 researchers from three organizations are collaborating in this experiment.

EXPERIMENTAL METHODS

Lake 382, typical of ELA lakes in size (37.7 ha) and bathymetry (13.1 m in maximum depth), was selected for its characteristic Precambrian Shield water chemistry and relatively large population of lake trout. The lake contains 2.2 mg/L dissolved calcium and has a hardness of 7.0 mg/L as calcium carbonate.

Starting in June 1987, the epilimnion of Lake 382 was annually loaded with Cd and 109Cd (0.00455 µg Cd/cpm) during the ice-free season. Target concentrations were 0.10, 0.10, 0.12 and 0.16 µg/L Cd in 1987 to 1990, respectively. The radioactive Cd was added to facilitate tracing the fate and accumulation of Cd. Most of the concentrations of Cd reported below are calculated from the [109Cd]. In the first four years, 975, 643, 778 and 1435 g of Cd were added. In 1990, depth profiles of [Cd] in the water were determined 6 times in the season. In March 1989, 23 sediment cores were taken along three transects radiating from the deepest point in the lake. To date, 10 cores have been analysed for [109Cd] and for [210Pb]. The 210Pb dating is used to estimate depth of the mixed layer in a core.

The fate (accumulation) of Cd has been measured in organisms from all of the trophic levels in Lake 382. Effects of Cd were and are being examined in respect to algal productivity, phytoplankton diversity and community structure, zooplankton community structure, mussel physiology (blood ionic composition, production of metallothionein) and fish structure and function. Lake 382 contains an estimated 750 lake trout (about twice as many as typical of ELA lakes of its size) along with about 2000 white suckers. These populations are large enough to sustain periodic sampling. Parameters examined in fish include:

1. Tissue uptake and distribution of Cd (PRIMARY RESPONSE): Levels of Cd in the vital organs are determined; these can then be related to physiological and pathological effects. The relationships derived from comparison between Cd burden and effects can then be used to predict consequences of similar Cd levels in other systems regardless of differences in water quality.

2. Biochemical and histological effects (SECONDARY RESPONSE):

   a. Carbohydrate metabolism (energy).
   b. Ionoregulation (maintenance of homeostasis).
   c. Metallothionein, acid-soluble thiols and ascorbate.

3. Whole-animal effects (TERTIARY RESPONSE):

   a. Growth indices.
   b. Reproductive indices.
   c. Behavioral responses.

4. Population and community effects (QUATERNARY RESPONSE):

   a. Population parameters: growth rates, survival, recruitment and abundance.
   b. Community parameters: population ratios, etc.

RESULTS - ABIOTIC COMPARTMENTS

The Cd loading to Lake 382 raised the mean measured [Cd] in the epilimnion from 0.0016 µg/L prior to Cd addition to 0.083, 0.050, 0.100 and about 0.150 µg/L, in 1987 to 1990, respectively. Cd water column profiles in 1990 reflected the seasonal changes in thermal stratification. Analysis of cores from March 1989 indicated that 73% (preliminary estimate) of the total Cd added in 1987 and 1988 was associated with sediments, but it is believed that the figure is closer to 90-95%. Estimates of amount of Cd in the sediments after additions of Cd in 1987 were 79-91%. 109Cd activity in deep sediments was evenly distibuted among cores, whereas in cores of shallow sediments, 109Cd activity depended upon their organic content. Sediments from below 7 m water depth are extremely liquid, 35-50% organic matter (loss on ignition) and show 109Cd mixing to as deep as 8-12 cm in the core. In the laboratory, a distribution coefficient between Cd and Lake 282 sediments was around 10^4.

RESULTS - FATE IN BIOTA

Plankton from 200 L of lake water from each thermal stratum were fractionated into >54 µm (primarily zooplankton) and 20-54 µm (primarily algae). In 1987 to 1989, the [109Cd] of the >54 µm fraction was consistently higher in the metalimnion than in the other lake strata until the fall samplings, when the [109Cd] were higher in the epilimnion. This indicates transport of organisms between the metalimnion and the epilimnion because little 109Cd reaches the lower strata until fall turnover. The 109Cd level of the plankton in the hypolimnion was low during most of the sampling season, but showed a slight rise in the fall prior to turnover. Normalized percentage of total water 109Cd associated with each size fraction were approximately 5% for the >54 µm fraction and up to 25% for the 20-54 µm fraction.

Accumulation of 109Cd in macrophytes in 1987, 1988 and 1989 was greatest in those producing a great deal of annual biomass (Nuphar, Potamogeton and Myriophyllum), and lowest in slow-growing perennials (Lobelia and other isoetids) which do not die back in the winter. Accumulation was very high in the roots of the emergent Carex, but the Cd was not transported to the leaves to a large extent. Water lilies may be an important route of Cd export to the terrestrial system. For example, they may be a source of the high Cd in the livers of moose which feed upon them.

The floater mussel, Anodonta grandis grandis accumulated 109Cd in the ice-free seasons of 1987 to 1990. By August 1990, mussels contained Cd at concentrations nearly 20 times that of pre-addition background levels and whole-body [Cd]:water [Cd] was approximately 15,000.

In 1989, [Cd] in whole fathead minnow and pearl dace were 8 to 15 times background levels and similar to those in 1987 and 1988. Concentrations of Cd in whole large fish (lake trout and white sucker) were 22 to 30 times background values by October 1989, and significantly higher than in 1988. The continuing accumulation in the longer-lived white sucker and lake trout probably indicates that they are not yet at equilibrium with the added Cd. The ratios of [Cd] in whole fish:water [Cd] were 2580, 2170, 890 and 680 for fathead minnow, pearl dace, white sucker and lake trout, respectively.

In 1989, trout and white sucker kidney Cd levels increased 5-10 fold over background despite Cd loading rate and water concentration similar to those in previous years. The large fish now have 4-5 μg Cd/g wet weight in kidney tissue. This represents concentration factors of greater than 40,000 over ambient water Cd levels. Histopathological lesions in rats begin when the level of Cd in the kidney exceeds 10 μg/g. Information for fish is limited, however, brook trout survival in a long-term laboratory exposure was reduced 50% when Cd concentration in kidney was 8 μg/g.

RESULTS - EFFECTS ON BIOTA

Lake 382 is seasonally more variable in its biological parameters than a number of other ELA lakes. In 1987 and 1988 there was approximately a 43% increase in annual mean chlorophyll _a_ concentration as compared to 1983 through 1986. The increase in primary production in 1988 was followed by a decrease in 1989. Reference lakes showed similar trends but the changes in Lake 382 were greater in magnitude. No effect on these parameters can be attributed to Cd without further monitoring.

Species diversity of phytoplankton in Lake 382 ranged between 0.80 to 0.90 from 1977 to 1988. In 1989, it was slightly lower at 0.78. This may still be within the range of natural variability or it may be an effect of the Cd. A decline in biomass (g/m^3) of phytoplankton in the epilimnion was observed in the spring and summer of 1989 and 1990, whereas fall biomass remained similar to a number of previous years. The possibility that there has been a change in phytoplankton species composition compared with that of reference lakes is being explored.

Experiments with enclosed _in situ_ zooplankton communities in Lake 382 before Cd was added and in an adjacent lake later, indicated that cladoceran species would be affected at 0.2 μg/L. In 1989, abundances in the total water column of rotifers, cladocerans, calanoid and cyclopoid copepods were higher than during prexposure (1985-1986) and in the first two years of Cd addition (1987-1988). However, the cladoceran, _Daphnia catawba_, less abundant than _D. galeata mendotae_, and occupying the metalimnion and hypolimnion, was less abundant in 1988 than in 1985 to 1987; in 1989 it was rare and found almost entirely in the hypolimnion. In 1989, the proportions of _D. galeata mendotae_ and of cladoceran eggs in the epilimnion compared with the whole water column were intermediate between the values seen before (1985-1986) and immediately after the first Cd additions (1987-1988). In 1987 and 1988 it appeared that _D. galeata mendotae_ was avoiding the epilimnion when [Cd] was high.

In July 1990, when epilimnetic Cd levels reached approximately 0.15 μg Cd/L, the usual large biomass of the >54 μm (primarily zooplankton) fraction was not found in the metalimnion. It was found in the hypolimnion where the Cd concentration was lower. This may be an indication of an avoidance reaction by the >54 μm zooplankton fraction.

By September 1989, metallothionein levels in mantle, gill, foot, kidney and visceral mass of mussels from Lake 382 were 2.1 to 3.8 times higher than in the body parts of mussels from uncontaminated reference Lake 377. The largest increases were in the gill, kidney and mantle.

Analysis of gut contents from Lake 382 lake trout so far indicates that these fish rely more heavily on invertebrates than do trout in reference Lake 468 and others. This coupled with their low growth rate suggests that trout in Lake 382 may be more susceptible to Cd-induced changes in invertebrate prey than populations from other lakes. Comparison of growth rates among Lake 382 and reference lakes indicates possible depressed early growth in white suckers in Lake 382.

In October 1987 and May/June 1988, all physiological parameters in fish from Lake 382 were similar to those in fish from reference lakes with the exception of liver glycogen which was initially 3-10 times higher in Lake 382 fish. Liver glycogen declined between July and September 1987 in trout from Lake 382, as did muscle protein. The decreasing energy reserves during this period may reflect changes in food supply or feeding behaviour. Biochemical parameters whose basal levels are diet-dependent (ascorbic acid and acid-soluble thiols) were also lower than levels found in fish from reference lakes. In 1989, biochemical analyses related to energy metabolism showed no remarkable differences between Lake 382 and reference systems. Plasma measurements assessing osmoregulatory function and carbohydrate metabolism were similar in fish from Lake 382 and in reference lakes for each year examined (1987-1989).

Metallothionein concentrations in liver and kidney of fish from Lake 382 varied seasonally but fell within the ranges found in fish from reference lakes.

Plasma thyroid hormones (T3 & T4) and thyroid epithelial cell height (TECH) were measured and compared to growth rates and gonad development in lake trout from Lake 468 and Cd-treated Lake 382. In 1989, plasma T3 exceeded T4 in fish from each lake. Indices of thyroid function showed pronounced changes during the open-water season only in fish from Lake 468. Fish in Lake 468 grew rapidly and T3 and TECH were inversely related to gonad development. Fish from Lake 382 had low plasma T3 and TECH which varied little during the open-water season. Elevated T3 and TECH were associated with active somatic growth and subsequent normal gonad development in Lake 468, whereas low T3 and TECH were associated with slow growth in Lake 382 fish. The difference in thyroid function between trout in Lake 382 and in reference lakes was evident early in 1987 prior to Cd additions and may be more related to differences in quality and quantity of food supply than to Cd. Growth rates of trout in Lake 382 are very slow and there is evidence that this was the case prior to Cd additions.

Information was gathered on variability ranges for ELA lake trout and white sucker egg parameters (differential counts, egg sizes profiles and gonadosomatic index) by sampling reference lakes along with Cd-treated Lake 382. The 1988 data showed a marked reduction in female lake trout gonadosomatic index in Lake 382 prior to spawning. A similar although less extensive effect was observed in nearby reference Lake 305. Also in 1988, vitellogenic oocytes were smaller than those found in fish from reference lakes (Lakes 468 and 305). Differential counts showed more recruitment eggs and fewer vitellogenic oocytes in September 1988 than in other years. Because variability in ovarian development has been attributed to the quantity and quality of food supply, alterations observed in 1988 appear related to reduced fish energy reserves (see above). In 1989, all egg and fecundity indices recovered and were similar to those found in 1987.

In the laboratory, preference-avoidance testing of previously unexposed lake whitefish to Cd measured a total of 27 response parameters on the behavioral responses of these fish to Cd ranging from 0.2 to 256 μg/L. While most fish showed "avoidance", some also displayed "preference" when measured simply as time spent on the treated side of the trough. A bimodal dose-response relationship was evident when the magnitude of the response and not the direction was considered. The fish reacted to water containing Cd at <1 μg/L and >8 μg/L but showed little reaction to intermediate concentrations. The fish responded to the lowest Cd concentration tested (0.2 μg/L), suggesting that their response threshold is lower still. Lake trout exposed to 0.5 and 5.0 μg/L Cd showed a dose-dependent decrease in their foraging capacity.

Long-term laboratory studies indicate that biochemical and histological effects occur when [Cd] reach approximately 8-10 μg/g in vital organs (liver and kidney) of lake trout. Biochemical responses were evident in more sensitive rainbow trout when tissue Cd levels were 2-4 μg/g.

CONCLUSIONS

1. Over 4 years, a total of 3.8 kg of Cd has been added to Lake 382. This has raised the concentration of Cd in the epilimnion up to 110 times background concentration. Most of the Cd added (at least 73%, but probably more than 90%) remains in the sediments. Although the lake is substantially contaminated by Cd, the water has not exceeded the Canadian Water Quality Guideline of 0.2 μg/L.

2. Cd has accumulated in all trophic levels; organisms now contain Cd up to 30 times background levels. Long-lived fish and mussels have not reached equilibrium with the added Cd and are still accumulating it.

3. There is no evidence of effects of Cd on primary production or nutrient cycles. There is no clear evidence of change in populations or communities of phytoplankton, zooplankton, mussels, crayfish or fish. However, phytoplankton species diversity may be declining and some zooplankters may be avoiding the high-Cd water. Based on in situ flow-through experiments on zooplankton, adverse effects are expected at 0.2 μg/L.

4. Lake trout in Lake 382 appear to rely more heavily on invertebrate prey than do trout in reference lakes. This coupled with their low growth rate would suggest that the trout may be more susceptible to declines in zooplankton than fish in other lakes.

5. Although there is yet no clear evidence of damage to the fish in Lake 382, kidneys of lake trout and white sucker contain Cd at about 50% of the concentrations producing histopathological lesions in rats.

6. We conclude that water quality guidelines alone are not sufficient to protect soft water systems from the effects of Cd because they do not prevent long-term accumulation of Cd in the sediments and biota. The question is not "Is the CWQG of 0.2 µg/L safe?" but "What sediment quality guidelines or restrictions on loading are required, as well, to protect freshwaters from persistent metals?"

MUSSELS AS BIOINDICATORS: A CASE STUDY OF TRIBUTYLTIN EFFECTS IN SAN DIEGO BAY

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ABSTRACT

As part of a Navy research program to evaluate the environmental effects of tributyltin (TBT) antifouling coatings and develop *in-situ* field bioindicators, native juvenile mussels were transplanted in San Diego Bay between 1987-1990. Serial seasonal transplants and intensive chemical sampling have documented temporal and spatial variability in TBT and the effects of TBT on growth, bioaccumulation and survival that have not been previously reported. Establishing this variability and identifying site-specific factors affecting juvenile mussel growth in San Diego Bay have facilitated environmentally relevant predictions of threshold levels for TBT effects on mussel growth. This represents the initial calibration of the mussel bioindicator for assessing TBT effects in San Diego Bay and establishes a significant refinement in the use of mussels as biological indicators. Based on the data presented here, questions have been raised regarding the environmental significance of the data and monitoring strategies that may be applicable to all bioindicator responses and monitoring programs.

INTRODUCTION

The first tributyltin (TBT)-based antifouling coatings were developed in 1961 but were not used in significant quantities for at least 10 years (Stebbing, 1985; Champ and Lowenstein, 1987) after demonstrating superior performance. TBT antifouling coatings are effective for up to 5 years compared to only 2 years for copper-based paints. By 1980 the U. S. Navy proposed painting its fleet with TBT antifouling coatings. An environmental assessment predicted an annual savings of $150,000,000 in fuel consumption and hull cleaning expenses and no significant adverse environmental impacts (U. S. Navy, 1985). Fleet implementation of TBT antifouling coatings was suspended because adverse effects on non-target organisms were observed with increased usage of TBT antifouling coatings in several European countries (U.S. Environmental Protection Agency, 1986). The use of biological indicators has played a significant role in decisions by state, federal and international regulatory agencies that have restricted the use of TBT antifouling coatings in the 1980's.

History of Regulations

In 1982 France became the first country to adopt restrictions on the use of TBT antifouling coatings based primarily on biological responses, i.e., abnormal shell thickening in the Pacific oyster *Crassostrea gigas* (Alzieu, 1986). In the U.K. restrictions were not imposed until 1986 after observations on shell thickening in *C. gigas* were supported by environmental measurements of TBT and associated with other adverse biological responses in laboratory studies (Abel et al., 1987). The U. S. Environmental Protection Agency (EPA) began a special review of TBT antifouling coatings in 1986 (U. S. Environmental Protection Agency, 1986), established an advisory in 1987 (U. S. Environmental Protection Agency, 1987) and Congress passed regulations in 1988 (U. S. Public Law, 1988). The State of California imposed restrictions on the use of TBT antifouling coatings in January, 1988 (State of California, 1988). The EPA advisory concentration for protecting marine life is 10 ng/L and in the State of California 6 ng/L. Regulations established by the State of California seem to have been unduly influenced by observations of shell thickening in *C. gigas* made by California Mussel Watch (Stephenson et al., 1986; Smith et al., 1987; Stephenson et al., 1988). The EPA special review also contains a requirement for an extensive monitoring and research program that includes bioindicators and chemical measurements to evaluate the effectiveness of the restrictions on the use of TBT. Most monitoring programs have been based on analytical measurements of TBT in water, sediment and tissues that do not directly measure biological effects. In addition, temporal and spatial variability generally have not been adequately measured for seawater TBT concentrations and the effects of TBT.

Biological Indicators

Although TBT antifouling coatings are designed to affect organisms that attach to ship hulls, the potential for environmental effects is significant among many non-target organisms. Molluscs seem particularly susceptible. Abnormal shell thickening in the cultured Pacific oyster *Crassostrea gigas* was first observed in the U. K. shortly after its introduction in 1976 and correlated with suspended sediment in preliminary studies (Key et al., 1976). Shortly thereafter the French discovered a correlation with shell thickening and proximity to marinas and the first association with TBT antifouling coatings (Alzieu, 1986). The regulation of TBT by the French preceded most other countries largely because of shell thickening on cultured oysters and before conclusive scientific evidence against TBT had been gathered (Salazar and Champ, 1988). This is an example of a biological indicator detecting potentially adverse environmental impacts before chemical detection. A biological indicator is the use of a biological response to quantify the presence of toxic chemicals in water and their effects on the biological environment.

Using biological indicators to assess and regulate environmental effects is becoming standard. Bioindicators have several advantages over water sampling and community studies for quantifying environmental levels of contamination and predicting environmental impact. Bioindicators provide integrated information about environmental conditions and bioavailability that cannot be defined with chemical sampling. In the last decade many regulatory requirements have shifted from chemical to biological measurements including survival, growth and bioaccumulation (Chapman, 1983; Chapman and Long, 1983; Phillips and Segar, 1986; Parrish et al., 1988). There has also been a shift toward site-specific bioassays and *in-situ* field testing. Waldock et al. (1990) make an important distinction between the use of biological indicators as detectors of environmental contamination by monitoring tissue accumulation versus their use as predictors of environmental impact by measuring other biological responses. Our field-transplant studies combined both approaches for a more integrated assessment.

Regulatory agencies must deal with the high degree of uncertainty in evaluating environmental effects and recommending appropriate environmental management practices, particularly with TBT (Salazar and Champ, 1988). There has been a tendency to emphasize bioindicators that are reportedly specific to TBT like shell thickening in *C. gigas*. Most of the evidence considered by the regulatory process to be most significant comes from bivalve molluscs. This has occurred for a variety of reasons. First, it is generally believed that molluscs are more sensitive than other animal groups to TBT, the primary toxic component of organotin antifouling paints. Second, many bivalves have a cosmopolitan distribution and are commonly maintained in the laboratory. Third, filter-feeding bivalves may be more susceptible to TBT due to their feeding strategy. Fourth, many bivalves have an economic importance in the commercial shellfish industry. In Europe, critical evidence was associated with shell thickening in oysters (*C. gigas*) (Alzieu, 1986; Waldock, 1986). In the U.S., the critical evidence was associated with three laboratory studies that reportedly demonstrated unacceptable effects on growth and development in oysters (*C. gigas*, *Ostrea edulis*) and clams (*Mercenaria mercenaria*) (U. S. Environmental Protection Agency, 1987).

Indicators of Contamination

Natural mussel populations have been used extensively as indicators of TBT contamination by measuring tissue concentrations of TBT in mussel tissues (Wade et al., 1988; Short and Sharp, 1989; Valkirs et al., 1990). Mussel transplants have been used similarly (Zuolian and Jensen, 1989). Bioaccumulation of TBT in mussel tissues is a potentially good indicator of environmental contamination because a single tissue measurement integrates long-term exposure to environmental concentrations of bioavailable TBT. Tissue measurements are therefore more environmentally meaningful than instantaneous, site-specific water measurements. However, bioavailable dose and actual exposure must be accurately quantified to definitively correlate water concentrations with tissue concentrations. Even then, accumulating levels of particular contaminants above ambient concentrations does not *a priori* indicate environmental impact to the bioindicator or other species (Peddicord, 1984). Using tissue accumulation in field bioindicators to quantify environmental levels of contamination is a potentially powerful tool, but there are many pitfalls involving interpretation and environmental significance (Phillips, 1980). Initial reports on mussels as bioaccumulators emphasized utility and minimized potential interference from extraneous environmental factors (Goldberg et al., 1978; Farrington et al., 1983; Goldberg et al., 1983). More recent reports have outlined potential problems in using tissue concentrations of contaminants for environmental prediction (Phillips, 1980; White, 1984; Phillips and Segar, 1986).

Indicators of Effects

Since bioaccumulation cannot be used to measure environmental effects directly, other biological responses (eg. growth, oxygen consumption, filtration rate) are often used in laboratory and field tests but there are similar difficulties in predicting environmental impact from these data (White and Champ, 1983; Cairns and Buikema, 1984; Malins et al., 1984; Moller, 1987; Cairns, 1988). There is a tremendous gap between correlations and causality when using biological indicators in the field, extrapolating from the laboratory to the field, or comparing laboratory data with field data. Specific problems with the interpretation and environmental significance of TBT data have been discussed previously (Stebbing, 1985; Salazar, 1986; Salazar and Champ, 1988; Salazar, 1989) as have the difficulties in extrapolating effects from shell thickening. Mussel growth represents the integration of biological responses to the environmental milieu, and reduced growth could have significant ecological consequences.

Both natural and pollution-related stresses have been shown to reduce mussel growth rates (Bayne et al., 1985). Reduced mussel growth has been associated with TBT in laboratory and field studies (Thain and Waldock, 1985; Stephenson et al., 1986; Salazar and Salazar, 1987; Stromgren and Bongard, 1987; Valkirs et al., 1987; Salazar and Salazar, 1988). Juvenile mussel growth was the most sensitive indicator of TBT measured in San Diego Bay microcosm experiments (Salazar and Salazar, 1987; Salazar et al., 1987). Threshold effects have been predicted from laboratory studies (Thain and Waldock, 1985; Thain, 1986, Thain and Waldock, 1986; Valkirs et al., 1987; Stromgren and Bongard, 1987), a portable flow-through field system in San Diego Bay (Salazar et al., 1987) and from mussel transplants in San Diego Bay (Stephenson et al., 1986; Salazar and Salazar, 1987, Salazar and Salazar, 1988; Salazar and Salazar, 1990a, Salazar and Salazar, 1990b). Most of the early laboratory studies and the earliest field study were conducted at very high test concentrations >100 ng/L, but since mean concentrations at all test sites in San Diego Bay are now <100 ng/L these early predictions are environmentally irrelevant. Juvenile mussels have particular advantages over adults as bioindicators: growth is not affected by gametogenesis (Rodhouse et al., 1986) and juveniles may be more sensitive to TBT than adults (Hall and Bushong, 1990). In addition, bioaccumulation in short-term tests with fast-growing juveniles more accurately reflects recent environmental changes (Fischer, 1983; Fischer, 1988).

U.S. Navy Monitoring Strategies

The Navy has a need to predict the environmental impact of a proposed program to paint their ships with TBT before implementation, and monitor effects thereafter. The Navy selected an integrated mussel monitoring approach because mussels are commonly used throughout the world and have been widely used in the U.S. and California. The objectives are similar to those of the California Mussel Watch program (Martin, 1985), i.e., to document and assess long-term trends in selected indicators of water quality, and provide data that are compatible with other monitoring programs. One part of the Navy monitoring program emphasizes many stations with quarterly monitoring of TBT in seawater, natural adult mussel tissues and sediment (Seligman et al., 1986; Seligman et al., 1990). This approach was part of a congressionally-mandated monitoring program whose main function was to document the extent of contamination through chemical measurements over time.

The work reported for our field-transplant studies is part of a Navy research program to develop biological indicators. We emphasize far fewer stations with more intensive serial sampling of TBT in seawater and mussel tissues as well as multiple biological measurements of transplanted juvenile mussels. Tissue TBT concentrations indicate the extent of contamination and mussel growth indicates environmental effects. Previously we discussed the use of mussels as bioindicators to determine the effects of TBT on survival, bioaccumulation and growth and established general relationships under natural conditions (Salazar and Salazar, 1990a). A model was developed to show the interactions between predicted threshold responses and various environmental variables. We have also discussed site-specific differences in TBT contamination and its effects and established temporal and spatial variability (Salazar and Salazar, 1990b). Predicted threshold responses were lowered based on site-specific effects.

Objective

The overall objective of this work was to examine the effects of TBT on mussels in San Diego Bay. More specific objectives included the following: 1) Document temporal and spatial variability in TBT concentrations and mussel responses; 2) Determine threshold response levels for concentrations of TBT in seawater and mussel tissues; 3) Calibrate and evaluate the utility of the mussel bioindicator. The main purpose of this paper is to discuss the environmental significance and monitoring implications of this work.

METHODS AND MATERIALS

Nine field-transplant studies were conducted for 12-week periods with natural populations of juvenile mussels (*Mytilus* sp.) in San Diego Bay between 1987 and 1990. One objective of these studies was to distinguish both the extent of TBT contamination and its effects among different sites. Intensive sampling over short time periods was conducted to detect differences. Serial seasonal transplant studies helped identify long-term trends. Details of these methods have been presented elsewhere (Salazar and Salazar, 1990a; Salazar and Salazar, 1990b).

Test Animals

Juvenile mussels (10-12 mm in length) were used to avoid the effects of gametogenesis on growth and because the literature suggested that juveniles are more sensitive than adults. Test mussels for these transplant studies were collected at the Magnetic Silencing Facility (MSF) just inside the mouth of the bay (Figure 1) where mean TBT concentrations in seawater and mussel tissues are among the lowest in San Diego Bay. Eighteen mussels were transplanted to each site at the beginning of each test. Test 1 animals were 10-15 mm in length ($\bar{x}$ = 12.0 mm). All others were 10-12 mm in length ($\bar{x}$ = 11.0 mm). Animals were continuously submerged either 1 m below the surface or 1 m above the bottom. Deep sites were included for comparative purposes because previous measurements in San Diego Bay marinas showed that seawater TBT concentration is often lower near the bottom (Seligman et al., 1986; Seligman et al., 1990). The main advantage of using transplants was the experimental control. Mussels can be transplanted to locations that require monitoring where they might not settle naturally. Effective settlement does not necessarily indicate healthy

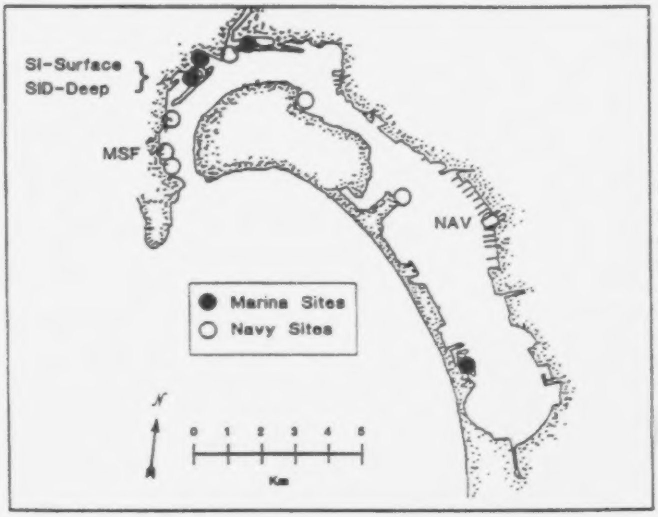


Figure 1. Juvenile mussel transplant sites in San Diego Bay. Marina (●) and Navy (○) sites are differentiated and four representative sites discussed in the text are called out. Two marina sites (SI, SID) are in the same location separated by 3 meters depth. The Navy sites are in the southern (NAV) and northern regions of the bay (MSF). MSF was also the mussel collection site.

growth conditions. Another advantage of caged mussels is multiple measurements made on individuals throughout the test period.

Measurements

Mussels were measured and water samples collected weekly during Tests 1-4 and on alternate weeks (biweekly) during Tests 5-9 after it was determined that weekly measurements reduced mussel growth rates (Salazar and Salazar, 1990a). Water samples collected at those frequencies were measured for chlorophyll-*a* and TBT concentrations. Current speeds were measured occasionally with an *in-situ* meter. Whole animal wet weights, lengths, shell weights, tissue wet weights, and tissue TBT concentrations were measured at the end of each study. Only weight growth rates will be discussed because there is a greater range of response than with length growth rates (25x compared to 10x), length measurements are less accurate, and plateaus are reached more quickly during each 12-week test. A 12-week test length was selected because 1) previous bioaccumulation rate measurements with adult mussels suggested equilibrium was reached in about 60 days, and 2) this was the approximate limit before our 10-12 mm juveniles matured and gametogenesis would affect growth rates.

Test Sites

San Diego Bay was selected for these field-transplant studies because of the high concentrations of TBT found in marinas there, the large size of the Navy fleet homeported there and proposed use of TBT by the U. S. Navy. Current EPA regulations restrict use of TBT antifouling coatings to vessels greater than 25 meters in length. However, due to widespread use on non-Navy vessels prior to 1988 and to vessels that still have TBT hull coatings, TBT persists in the environment at concentrations predicted to cause adverse effects. Monitoring sites were selected primarily for the greatest range of seawater TBT concentrations and other environmental conditions although access and security were also important. The 18 transplant sites extended from the northern to southern regions of San Diego Bay (Figure 1) and included seven marina sites and 11 Navy sites. Marina sites within enclosed yacht basins were selected because previous studies have shown that the highest concentrations of seawater TBT are generally associated with high densities of TBT-coated vessels in basins with poor flushing (Seligman et al., 1986). The relatively narrow entrances to these basins and other restrictions to flushing are shown in Figure 1. Navy sites were monitored because TBT antifouling coatings are not generally used on Navy vessels and previous monitoring studies indicated sediment and seawater TBT concentrations were quite low in these areas (Seligman et al., 1990). It was important to collect baseline information and use those data to predict environmental effects before the Navy could use TBT antifouling coatings.

The general relationships between seawater TBT, tissue TBT and growth rates have been discussed previously (Salazar and Salazar, 1990a) and comparisons between marina and Navy sites have been made (Salazar and Salazar, 1990b). To gain a better understanding of the threshold effects of TBT and the environmental significance of mussels as bioindicators, specific comparisons are made in this paper between two representative marina sites (SI, SID) and two Navy sites (NAV, MSF). The SI surface site (1 meter below the surface) and SID deep site (1 meter off the bottom) are located in the back of the Shelter Island Yacht Basin; a small area with very restricted flushing (Figure 1) that accommodates at least 2,200 vessels. Water depth is approximately 5 meters. Seawater concentrations of TBT in this basin are currently the highest in San Diego Bay and were previously among the highest ever reported

(Valkirs et al., 1986). These high seawater TBT concentrations have been shown to vary by more than an order-of-magnitude with tidal cycle (Clavell et al., 1986; Stang et al., 1989). The Magnetic Silencing Facility (MSF) is near the mouth of San Diego Bay. It is characterized by good flushing, relatively uncontaminated water, coastal ocean temperatures and very few vessels. Water depth is approximately 5 meters and the site is influenced by both ocean and bay water. MSF is the site least influenced by activities in the bay and bay geography. Naval Station San Diego (NAV), is in the southern end of San Diego Bay and is characterized by many large vessels adjacent to rows of long piers associated with high activity and many support operations. Flushing is suppressed, temperatures are elevated and chlorophyll-*a* concentrations are low. Water depth is approximately 12 meters. Although seawater TBT concentrations are low there, we have measured other contaminants at high concentrations. NAV is the most southern Navy site and exemplifies the extremes of bay conditions.

Data Analysis

Mean growth rates and seawater TBT concentrations for each site in each test were estimated from linear regressions on weekly or biweekly measurements. Each data point for mussel growth and tissue accumulation represents a 12-week mean for 18 animals. Graphical methods were used to display the general relationships among environmental levels of seawater TBT, tissue accumulation of TBT, and mussel growth. The statistical significance of each relationship was determined from linear regression analyses. Linear regressions were also used to determine the significance of long-term trends. In the time-series data, an Analysis of Variance (ANOVA) was used to determine differences between sites by test and across tests for seawater TBT concentrations, growth rate, temperature, and chlorophyll-*a*. Relationships were considered statistically significant at the 95% confidence level. Error bars represent ± two standard errors. Since tissue TBT concentrations are represented by single, end-of-test measurements, only differences across tests are compared statistically and no error term can be associated with individual test measurements. To demonstrate the extreme variability and differences in temperature, chlorophyll-*a* and seawater TBT concentrations at the two marina and two Navy sites, graphs have been produced from the individual data points rather than from mean values. Several comparisons were made between pooling all data, pooling data by region (marina vs Navy) and individual site data to assess the environmental significance and statistical validity of pooled data and ramifications for environmental monitoring programs.

RESULTS

General Trends

Mean 12-week concentrations of seawater TBT ranged from 2 to 530 ng/L and mean 12-week growth rates from 17 to 505 mg/wk (0.2 to 2.4 mm/wk). Transplanted juvenile mussels at every site accumulated measurable amounts of TBT during each test. Tissue concentrations of TBT ranged from 0.1 to 3.2 μg/g wet weight. The majority of mussels transplanted at marina sites accumulated TBT at concentrations >1 μg/g while mussels transplanted at Navy sites generally accumulated <0.5 μg TBT/g. These tissue TBT concentrations corresponded to seawater concentrations near 100 ng/L and above in marinas and <10 ng/L at Navy sites. Only at Navy sites adjacent to marinas were mussel tissue TBT concentrations >0.5 μg/g. The relationship between seawater TBT and growth is better than that for tissue TBT and growth. The lowest growth rates were associated with the highest concentrations of seawater TBT, temperatures near 14.5°C, and low chlorophyll-*a* concentrations. The highest growth rates were associated with low concentrations of TBT, high chlorophyll-*a* concentrations and low temperatures near 20°C. There are significant relationships between seawater TBT and tissue TBT. Growth rate is significantly related to both seawater TBT and tissue TBT. There is a statistically significant difference in seawater TBT, tissue TBT and mussel growth rate when marina sites are compared to Navy sites.

Seawater TBT & Tissue TBT

There is a significant linear relationship between seawater TBT and tissue TBT (Figure 2). However, this relationship is different for lower seawater TBT concentrations than higher ones. The slope of the regression for seawater TBT concentrations $\leq$105 ng/L is almost 5 times higher than the slope at seawater TBT concentrations >105 ng/L and suggests a higher bioconcentration factor at lower seawater TBT concentrations. The highest seawater and tissue concentrations of TBT were found in marinas. The majority of seawater TBT concentrations were <100 ng/L and tissue TBT concentrations <1 µg/g wet weight. Six of the seven highest seawater and tissue TBT concentrations were measured in the Shelter Island Yacht Basin (SI, SID). These data strongly influence the relationship between seawater TBT and tissue TBT shown in Figure 2.

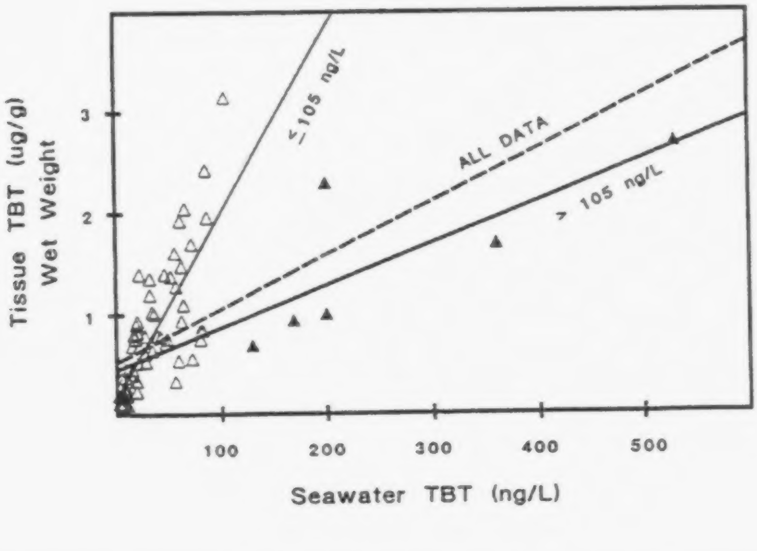


Figure 2. Positive linear relationship between TBT concentrations in seawater and mussel tissue. Seawater numbers are 12-week means and tissue numbers are end-of-test measurements. Regression lines show differences in tissue TBT accumulation between low (<105 ng/L) and high (>105 ng/L) seawater TBT concentrations and the regression for all data.

TBT & Mussel Growth

There is a significant negative exponential relationship between juvenile mussel growth and tissue TBT concentration. Figure 3 shows the high degree of variability in growth at all tissue TBT concentrations. Based on regression analyses, juvenile mussel growth rate is less dependent on tissue TBT concentration than seawater TBT concentration. Only 14% of the variance in growth can be explained by tissue TBT concentration. This suggests that the toxicity of TBT in seawater has a more direct effect on mussel growth than TBT accumulated in mussel tissues. There are no significant regressions when the analyses are limited to data <1.5 $\mu g/g$. Based on analytical measurements, field observations and statistical analysis, the threshold level of tissue TBT concentration for probable effects on mussel growth was estimated at 1.5 $\mu g/g$ and the no-effect level at 0.5 $\mu g/g$. There is a zone of uncertain effects between.

There is a significant negative exponential relationship between juvenile mussel growth and seawater TBT concentration (Figure 4). Based on regression analysis, 51% of the growth variance can be explained by seawater TBT concentration. Six of the seven highest seawater TBT measurements and five of the six lowest growth rates were measured in the Shelter Island Yacht Basin (SI, SID). As seawater TBT concentrations decreased, mussel growth rates for mussels transplanted at SID were among the highest measured at all sites. The seven data points for seawater TBT concentrations >100 ng/L strongly influence the significance of the regression for all data. However, the equations describing the relationships for growth rate at high and low seawater concentrations of TBT are quite similar. There is also a significant exponential relationship when the seawater TBT data <100 ng/L are analyzed separately, but in this case only 11% of the growth variance can be explained by seawater TBT concentration. The lowest seawater TBT concentration that resulted in a statistically significant relationship was near 70 ng/L. Environmental effects are uncertain, however, since only 7% of the growth variance can be explained by seawater TBT concentrations. Under the most adverse conditions, growth could be affected by lower concentrations. Based on analytical measurements, field observations and statistical analysis, the threshold level of seawater TBT concentration for probable effects on mussel growth was estimated at 100 ng/L and the no-effect level at 25 ng/L. There is a zone of uncertain effects between.

TBT & Effects: Marina vs Navy

There is a statistically significant difference in seawater TBT, tissue TBT and mussel growth rates across tests when marina sites are compared to Navy sites. Figure 5 shows that the mean concentration of seawater TBT in marinas has declined rapidly since 1987 and by October, 1989, approached the mean for Navy sites. Seawater TBT and tissue TBT concentrations at marina and Navy sites were significantly different in every tests and growth rates were significantly different in most. Pooling sites suggests that seawater and tissue TBT concentrations decreased while mussel growth rates increased over time. However neither tissue TBT concentrations or growth rates consistently tracked seawater TBT concentrations. Tissue TBT concentrations were highly variable and growth rates increased steadily until Test 8 when a sharp decline was associated with winter seawater temperatures $<15°C$.

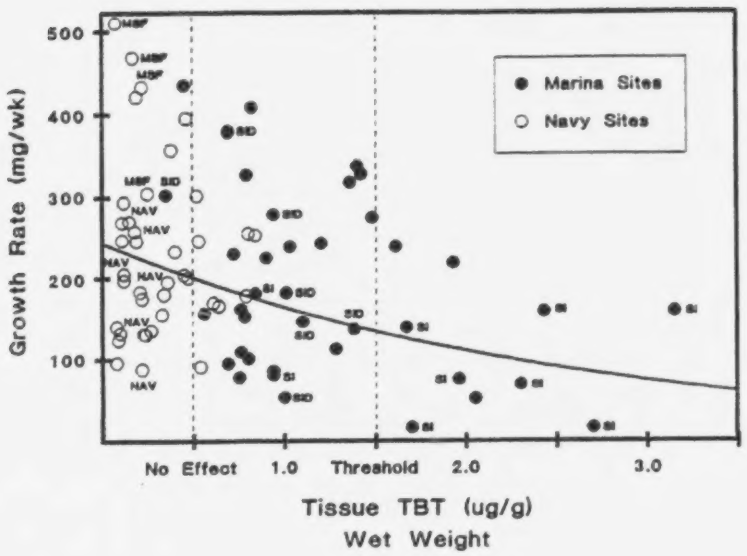


Figure 3. Negative exponential relationship between tissue TBT concentration and juvenile mussel growth rate. Marina (●) and Navy (○) sites are differentiated and the four representative sites discussed in the text are identified (SI, SID, MSF, NAV). Predicted No Effect and Threshold Effect concentrations are shown by the dotted vertical lines.

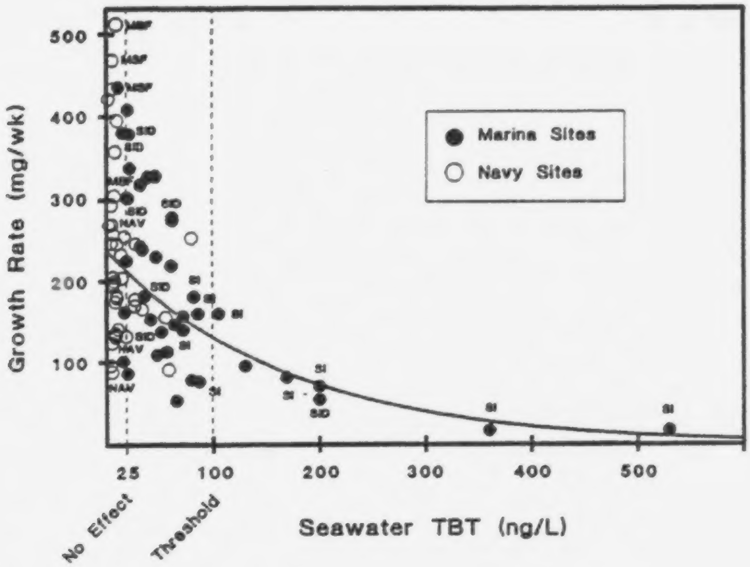


Figure 4. Negative exponential relationship between seawater TBT concentration and juvenile mussel growth rate. Marina (●) and Navy (○) sites are differentiated and the four representative sites discussed in the text are identified (SI, SID, MSF, NAV). Predicted No Effect and Threshold Effect concentrations are shown by the dotted vertical lines.

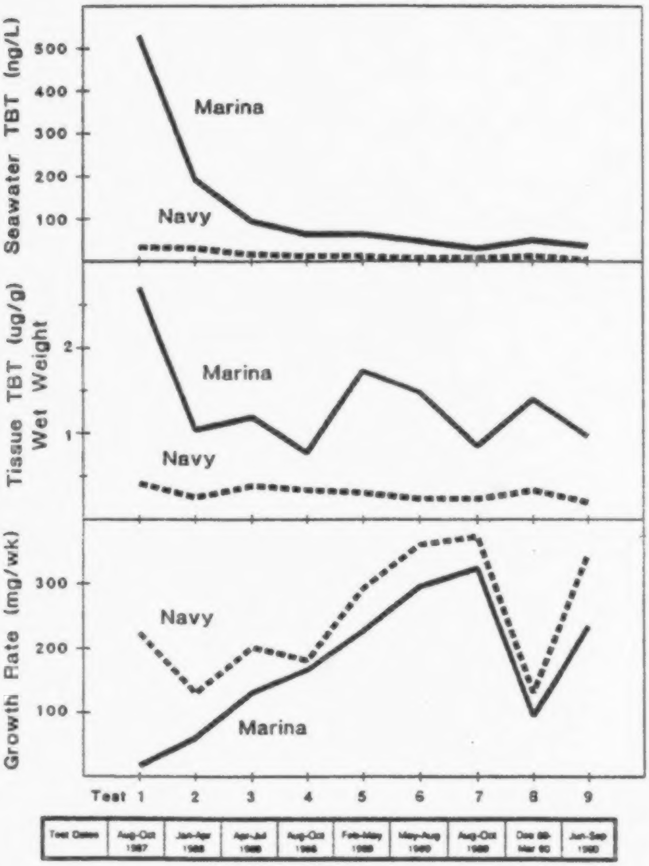
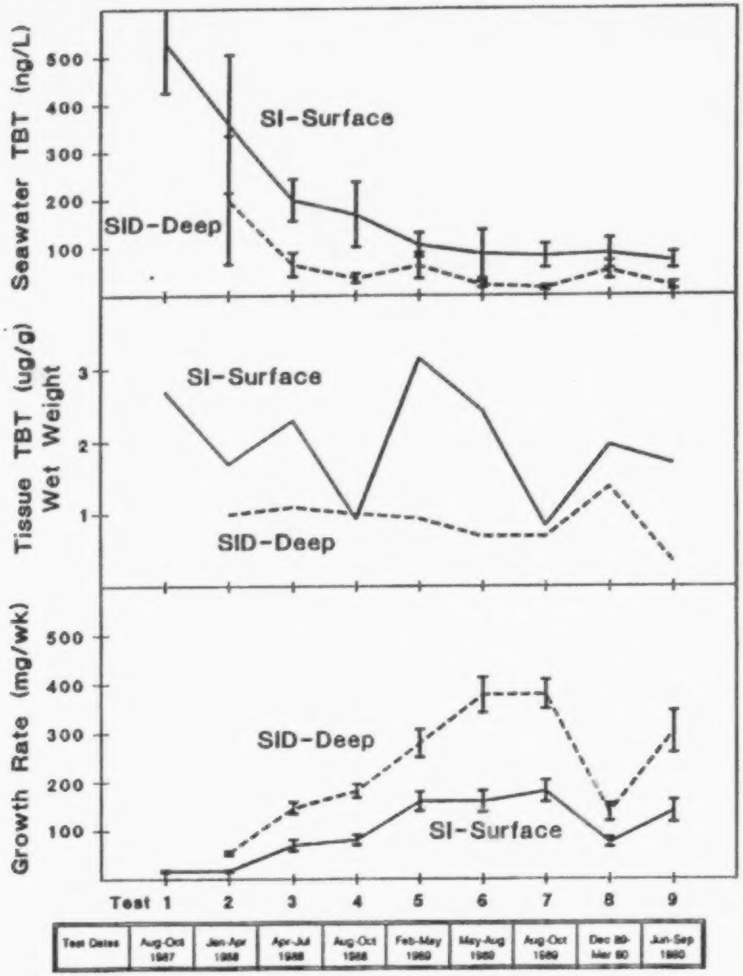


Figure 5. Temporal and spatial differences in mean seawater TBT (ng/L), tissue TBT (μg/g) wet weight, and growth rate (mg/wk) for marina (—) and Navy (- - -) sites. Also given are test number and corresponding dates.

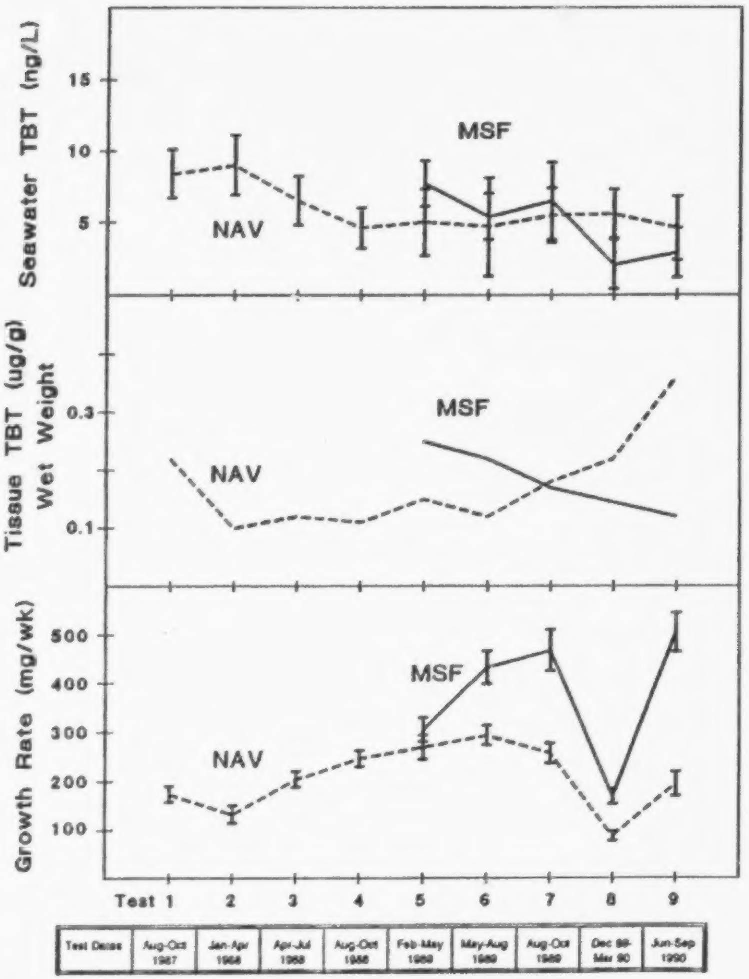
TBT & Effects: Marina Sites

The lowest growth rates measured in San Diego Bay were 17 mg/wk at SI in both Tests 1 and 2. There is a statistically significant difference in seawater TBT concentration, tissue TBT concentration, and juvenile mussel growth rates when SI is compared to SID (Figure 6). These sites, located in the Shelter Island Yacht Basin, are separated by a vertical distance of only 3 meters. There was a dramatic decrease in seawater TBT at both sites from 1987 to 1990. Seawater TBT concentrations at the surface site (SI) were always higher than at the bottom site (SID). The seawater TBT concentrations at SI are approaching those at SID, but they are still significantly different. Tissue TBT concentrations did not consistently follow seawater TBT concentrations, particularly at SI where seawater TBT concentrations were the highest. Growth rates for mussels transplanted at SID were significantly higher than at SI. After Test 4 growth rates at SID were significantly higher than for mussels transplanted to most Navy sites, even though seawater TBT concentrations were significantly higher at SID. These individual site comparisons suggest that while seawater TBT



Test Dates	Aug-Oct 1987	Jan-Apr 1988	Apr-Jul 1988	Aug-Oct 1988	Feb-May 1989	May-Aug 1989	Aug-Oct 1989	Dec 89-Mar 90	Jun-Sep 1990

Figure 6. Temporal and spatial differences in mean seawater TBT (ng/L), tissue TBT ($\mu g/g$) wet weight, and growth rate (mg/wk) for marina sites SI (—) and SID (- - -) in the Shelter Island Yacht Basin. These sites are separated by only 3 meters depth. Test numbers and corresponding dates are also given. Error bars represent ± 2 standard errors.



Test Dates	Aug-Oct 1987	Jan-Apr 1988	Apr-Jul 1988	Aug-Oct 1988	Feb-May 1989	May-Aug 1989	Aug-Oct 1989	Dec 89-Mar 90	Jun-Sep 1990

Figure 7. Temporal and spatial differences in mean seawater TBT (ng/L), tissue TBT ($\mu g/g$) wet weight, and growth rate for Navy sites MSF (—) in the north bay and NAV (- - -) (Naval Station San Diego) in the south bay. Test numbers and corresponding dates are also given. Error bars represent ± 2 standard errors.

concentrations decreased and growth rates increased, tissue TBT concentrations did not change significantly.

TBT & Effects: Navy Sites

NAV is the only Navy site that has been monitored in every test. At this site seawater TBT concentration has decreased, tissue TBT concentration has increased, and growth rate followed a seasonal cycle (Figure 7). The MSF site was monitored from Tests 5 through 9. During this time seawater TBT concentration decreased, tissue TBT concentration decreased and growth rates also followed a seasonal cycle. The highest juvenile mussel growth rate we ever measured in San Diego Bay, 505 mg/wk, was recorded at MSF in Test 9. In the tests where NAV and MSF could be compared, there were no statistically significant differences in seawater TBT concentrations or tissue TBT concentrations. There was a statistically significant difference in juvenile mussel growth rate. Seawater TBT concentrations and tissue TBT concentrations were much lower at the Navy sites than at the marina sites. Growth rates were also higher for mussels transplanted at the Navy sites, except during Tests 5-9 when growth rates for mussels transplanted at SID were higher than those at NAV.

Natural Variability in Seawater TBT

Some general trends in environmental concentrations of TBT, natural factors and their association with mussel growth can be more easily identified by pooling data for all stations by test, even though this procedure may be statistically inappropriate. The seasonal variability in temperature, chlorophyll-*a* and growth rate, and their relationship to seawater TBT concentrations and tissue TBT concentrations are shown in Figure 8. There was a rapid decline in seawater TBT concentration during the first three tests that was probably unrelated to seasonal factors. This was followed by a more gradual decrease in seawater TBT concentration with some intermittent increases that could be related to seasonal factors. Tissue TBT concentration seems to be associated with seasonal factors and mussel growth rate in addition to seawater TBT concentrations. There are seasonal cycles of chlorophyll-*a*, growth and temperature which covary by test and season. The lowest growth rates are associated with the lowest temperatures and the lowest concentrations of chlorophyll-*a*.

The fine structure of this temporal and spatial variability is shown in Figure 9 where the two marina sites (SI, SID) and two Navy sites (MSF, NAV) are compared with respect to seawater TBT concentrations, chlorophyll-*a* and temperature. The main purpose of this figure is to demonstrate the tremendous variability in these three factors over time but overall differences are also apparent. Although we found a statistically significant difference across tests between SI and SID temperatures, they are very close and the large number of data points largely obscure the subtle differences we found. It may be inappropriate to use mean values for these factors because it is not clear whether mussels are responding to means or extremes. At high seawater TBT concentrations we measured variability approaching an order-of-magnitude during the 12-week exposure periods. Chlorophyll-*a* was generally much lower in the winter than in the spring and summer. Chlorophyll-*a* concentrations were significantly higher at SID than SI, and significantly higher at MSF than NAV. Temperatures at NAV were generally much higher than MSF, but they were similar during the winter. Daily temperature variation was much greater at MSF than NAV. Similar differences in temperature variability were found between SI and SID, albeit not as dramatic.

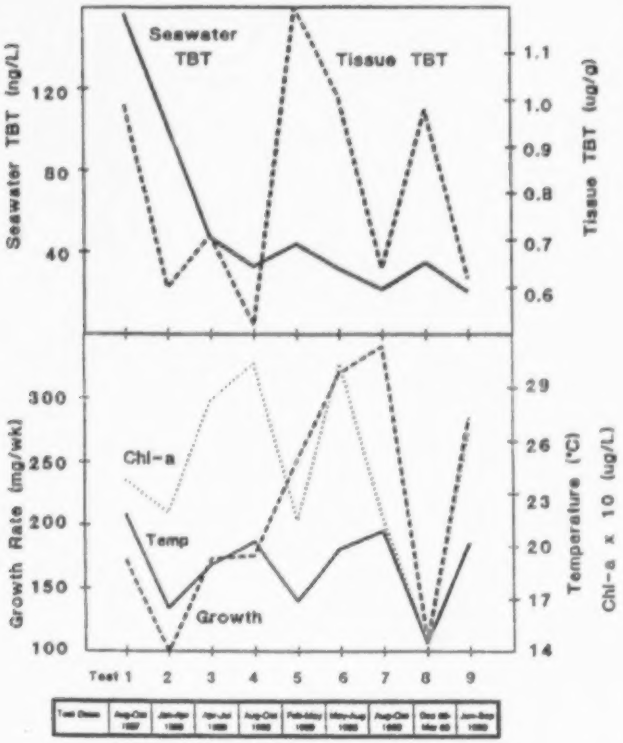


Figure 8. Mean seasonal variability in seawater temperature (°C), chlorophyll-a (μg/g) and juvenile mussel growth in San Diego Bay between 1987-1990. Mean seawater TBT (ng/L) and tissue TBT (μg/g) wet weight are also shown.

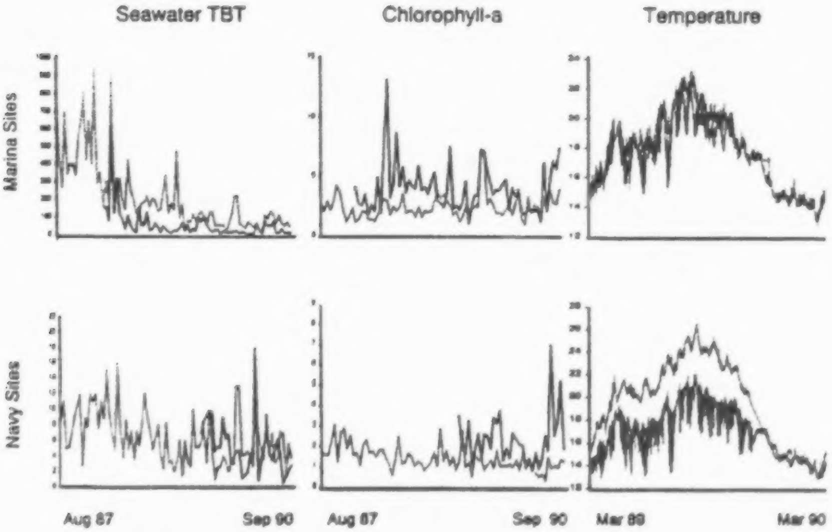


Figure 9. Comparison of temporal and spatial variability in seawater TBT (ng/L), chlorophyll-a (μg/g), and temperature (°C) between two representative marina sites (SI - - -, SID —) and two representative Navy sites (NAV - - -, MSF —) between August 1987 - September 1990 (March 1989 - March 1990 for temperature).

There are three important differences in these parameters to be distinguished here that may have biological relevance. They are mean values, daily variability and seasonal changes. Mean values show statistically significant differences in seawater TBT concentrations between SI and SID but no differences between NAV and MSF. Mean values show statistically significant differences in both chlorophyll-*a* and temperature when comparing SI and SID as well as between NAV and MSF. Although seasonal variability is similar for each parameter and each site, the differences in daily or short-term variability are dramatic. The most dramatic differences were detected with *in-situ* temperature monitors that recorded temperature at half-hour intervals during the entire test period. Although mean temperatures are significantly higher at NAV, short-term variability at MSF was significantly higher.

DISCUSSION

We will discuss long-term trends in seawater TBT and tissue TBT and their effects on mussel growth, threshold responses predicted from these data, the environmental significance of short-term variability in natural factors, and the monitoring implications of the work. Several comparisons will be made to the Navy statutory monitoring program (Seligman et al., 1990, Valkirs et al., 1990). It should be noted that our bioindicator program and the monitoring program were both sponsored by the Navy and conducted in the same laboratory at the Naval Ocean Systems Center, therefore all the measurement techniques are comparable. The purpose of our program was to develop an *in-situ* field bioindicator system to assess the environmental impact of Navy contaminants. We measured survival, growth and bioaccumulation of TBT in transplanted juvenile mussels to establish the extent of contamination and resulting effects. The monitoring program measured TBT in water, sediment and natural adult mussel tissues to establish an environmental baseline for TBT and document long-term trends.

Marina vs Navy Sites

A statistically significant difference in seawater TBT concentration, tissue TBT concentration and mussel growth rates was found for the test period 1987-1990 when Navy sites were compared with marina sites. The decrease in seawater and tissue TBT concentrations for both marina and Navy sites was similar to that reported by Valkirs et al. (Valkirs et al., 1990) for Navy statutory monitoring during the same time period. It is instructive to emphasize the similarities and differences since the monitoring program sampled ~ 25 sites on a quarterly basis, whereas in our bioindicator approach we sampled ~ 10 sites per test either weekly or biweekly for periods of 12 consecutive weeks over the 3-year test period. Both approaches produced similar results for pooled regions, but our intensive short-term sampling and use of transplanted mussels permitted site-specific distinctions to be identified that are not possible with long-term monitoring of natural populations. The precipitous decline in seawater TBT concentrations at marinas coincides with restrictions on the use of TBT antifouling coatings by the State of California in January, 1988 (State of California, 1988) and demonstrates the effectiveness of those restrictions. A similar but less dramatic decrease in seawater TBT concentration for Navy sites suggests that marinas are the primary source of TBT at all Navy sites and for the entire bay as previously reported by Seligman et al. (1986, 1990).

General trends can be misleading however, and pooling data from different monitoring stations can mask important differences and bias the mean values. Although tissue TBT concentrations in our study decreased at marina and Navy sites when pooled, some sites did not change significantly while others either increased or decreased in tissue TBT concentrations. These apparent anomalies suggest that factors other than seawater TBT concentration may be affecting TBT uptake in mussels. Increases in juvenile mussel growth rates were considered significant and related to lower seawater TBT concentrations and reduced handling as previously reported (Salazar and Salazar, 1990a). We have also suggested how growth rates can affect bioaccumulation and therefore natural factors that affect growth rates can indirectly affect the bioaccumulation process.

Examining these trends in seawater and tissue TBT concentrations and mussel growth rates on a site-by-site basis reveals the fine structure of variability and helps define the advantages and limitations of this approach for environmental prediction. Although the statutory monitoring program gives a better overall chemical description of San Diego Bay by monitoring 25 sites within four regions, a single quarterly sample per site has no associated error term and cannot define short-term variability at a given site. We are able to associate an error term estimating variability in both seawater TBT concentrations and mussel growth rate with respective 12-week means and establish confidence limits on that number. Additionally, only the growth data provide a direct measure of environmental effects.

Marina Sites: SI vs SID

There are statistically significant differences in seawater TBT concentrations, tissue TBT concentrations and mussel growth rates in every test where the Shelter Island Yacht Basin surface (SI) and deep (SID) sites are compared. This is surprising since these two sites are separated by a vertical distance of only 3 meters. The differences show how pooling data for these sites could be misleading. The higher concentrations of both seawater and tissue TBT at the SI site (1 meter below the surface) compared to the SID site (1 meter off the bottom) also imply that ship hulls are the primary source of seawater TBT rather than TBT desorbed from contaminated sediment. This is consistent with sediment desorption measurements in San Diego Bay (Kram et al., 1989) and bioassays performed on TBT contaminated sediment from Commercial Basin (Salazar and Salazar, 1985). This particular sediment was the most contaminated in San Diego Bay (Valkirs et al., 1990) and yet there was no apparent toxicity from the very high levels of TBT measured in the sediment.

Higher growth rates suggest that mussels transplanted to SID near the bottom remained healthier than those transplanted near the surface. In addition to seawater TBT concentrations being lower at the bottom, water temperatures were lower and chlorophyll-*a* concentrations were higher. The added effect of these environmental factors increases the difficulty in separating the beneficial effects of low summer temperature and better nutrition from the adverse effects of TBT. A seasonal thermocline has been identified in the Shelter Island Yacht Basin (Stang et al., 1989) resulting in two very different water masses that could account for the differences in seawater TBT concentrations measured here and reported by others (Seligman et al., 1986; Stang et al., 1989). Tidal influence in San Diego Bay is very strong and order-of-magnitude changes in seawater TBT concentrations occur with the tidal cycle in the vicinity of the Shelter Island Yacht Basin (Clavell et al., 1986; Stang et al., 1989). Similar variability with tidal cycle has been reported for other contaminants as well (Zirino et al., 1978). All of these findings identify the surface site SI as being environmentally different than the bottom site SID. Such physical, chemical and biological differences argue against pooling data. They also show that additional physical and chemical parameters must be measured in monitoring studies to quantify environmental factors that could account for observed differences in juvenile mussel growth and bioaccumulation.

Navy Sites: NAV vs MSF

There were no statistically significant differences in seawater TBT concentrations and tissue TBT concentrations when the Navy sites NAV and MSF were compared. However, juvenile mussel growth rates for NAV transplants are significantly lower than growth rates for MSF transplants. Seawater TBT concentration was not a significant factor in regulating mussel growth rates at Navy sites because seawater TBT concentrations at most Navy sites were <10 ng/L. The physical, chemical and biological conditions present at each of these sites were quite different and probably responsible for the observed differences in juvenile mussel growth. MSF is near the mouth of the bay where flushing is good and concentrations of most contaminants are low. NAV is located in the southern portion of San Diego Bay. It is characterized by high summer temperatures near 25°C and low chlorophyll-*a* concentrations near 1 $\mu g/g$. We have also measured high concentrations of heavy metals and petroleum hydrocarbons in both seawater and mussel tissues at NAV. This is not surprising since the Naval Station supports many large vessels. This also suggests why chemical monitoring of a single contaminant should not be used for environmental prediction.

Further, chemical monitoring of TBT in seawater, sediment, and mussel tissues does not identify natural environmental stress factors or stress from other contaminants. Biological effects can only be inferred by comparing environmental levels of TBT with levels found to cause effects in laboratory studies. Biological monitoring can indicate effects without identifying the source. This has significant ramifications for the use of monitoring programs to determine threshold responses of contaminants and predict environmental significance. To determine the actual effects of TBT, other factors must be measured and evaluated before using correlations as cause-effect relationships. NAV (Naval Station San Diego) is a particularly important site because a high density of large vessels coated with TBT would be located here if application was approved. Since the chemical sampling of the statutory monitoring program did not measure biological effects and since we only had one Naval Station site (NAV), neither monitoring program can accurately document the biological environment at Naval Station San Diego or predict the environmental impact of coating the Navy ships there with TBT. These two monitoring programs could be combined and expanded to monitor other factors that would facilitate more accurate environmental predictions.

Threshold Responses

Seawater TBT, tissue TBT and mussel growth rates have been used to distinguish site-specific differences in contamination and effects (Salazar and Salazar, 1990b). Here, we have examined these relationships in more detail. Based on mussel growth rates and other factors, the threshold level of tissue TBT concentration for probable adverse effects on mussel growth is estimated at 1.5 $\mu g/g$ and the no effect level at 0.5 $\mu g/g$. Between is a zone of uncertain effects. This threshold tissue TBT level is similar to one predicted from scope-for-growth studies (Page and Widdows, 1990) and oyster growth studies (Waldock et al., 1990). The threshold response level for seawater TBT is predicted at 100 ng/L and the no effect level at 25 ng/L. Between is a zone of uncertain effects. The threshold level is similar to the threshold predicted for effects on oyster growth. The no effect level is similar to the original regulatory level established of 20 ng/L in the U.K. (Abel et al., 1986) and the EPA advisory of 10 ng/L in the U.S. (U. S. Environmental Protection Agency, 1986). Although our data can be used to support these regulatory levels, we still believe the regulatory process is flawed (Salazar and Champ, 1988), and regulators may have made the right decision for the wrong reasons by using laboratory toxicity tests alone to establish regulatory criteria (White and Champ, 1983). We have demonstrated that restrictions on the use of TBT antifouling coatings have improved mussel growth in San Diego Bay, particularly in marinas and areas adjacent to marinas. This suggests that the field bioindicator approach is valid and that the regulatory process should include a similar field validation approach.

Mussel growth can be an effective and dynamic indicator of environmental stress because it represents an integrated response of biological processes to the environment. The first reported effects of TBT on juvenile mussel growth at seawater TBT concentrations < 100 ng/L were recorded in our flow-through microcosm in San Diego Bay (Salazar et al., 1987). Although we found statistically significant effects at seawater TBT concentrations near 70 ng/L, it was suggested that threshold effects were overestimated based on temperature and nutritive stress induced by the test system. After the first three San Diego Bay juvenile mussel transplant studies, significant effects of TBT on mussel growth were reported at seawater concentrations > 200 ng/L and it was suggested that natural factors modified TBT effects at concentrations < 100 ng/L (Salazar and Salazar, 1988). The general relationships from nine transplant studies also estimate threshold effects at 100 ng/L (Salazar and Salazar, 1990a). It was acknowledged that effects could occur at lower concentrations if the animals were under stress from other environmental factors. Statistically significant effects were found near 70 ng/L in this study as well, but the prediction of these effects is highly uncertain when extrapolating to natural conditions. The site-specific differences in our most recent work allowed detection of no effect levels and threshold levels for effects of seawater TBT and tissue TBT (Salazar and Salazar, 1990b). We have shown how the general relationships previously established by statistically significant regressions can be misleading unless sufficient site-specific data are reported. The threshold values for effects of seawater TBT and tissue TBT concentrations on juvenile mussel growth rates were adjusted downward to reflect site-specific effects. In other words, we associated an error term with the predicted thresholds. Statistical significance often differs from environmental significance. It appears that growth is a response to site-specific effects that incorporate the effect of extreme conditions as well as long term means.

There is a significant positive linear relationship between seawater TBT and tissue TBT concentrations but the relationship changes with seawater TBT concentrations > 100 ng/L. The slope of the linear regression is approximately 5 times greater when only seawater TBT concentrations < 100 ng/L are used. We have previously suggested that seawater TBT concentrations could not be predicted from tissue TBT concentrations. While this may be generally true, since 12-week mean concentrations of seawater TBT at all our San Diego Bay sites are now < 100 ng/L, the estimates are better. Even so, tissue TBT concentration can still probably only be used to detect approximate order-of-magnitude differences as originally intended in a Mussel Watch approach (Goldberg et al., 1978). This has significant implications for interpreting data from any chemical monitoring program based on measuring tissue concentrations of various contaminants for predictive purposes. Many investigators tend to over-extend the utility of tissue accumulation data and fail to discuss the pitfalls in applying these data for predictive purposes (Phillips, 1980).

Factors Modifying Threshold Responses

White (1984) has cautioned against the arbitrary use of mussel monitoring systems without developing a model to be tested. We have developed a mussel bioindicator model that emphasizes the importance of natural factors and other contaminants on mussel survival, bioaccumulation and growth (Salazar and Salazar, 1990a). Although there is a correlation between decreases in seawater TBT and increases in juvenile mussel growth rates, there are other factors that also regulate mussel growth. The extreme drop in growth rates between December 1989 and March 1990, when seawater TBT concentrations were quite low, suggests that temperature was a significant factor. The seasonal effects of temperature and nutrition on mussel growth rates are well known (Seed, 1976; Newell, 1979) and we have previously discussed their effects in detail (Salazar and Salazar, 1990a). The adverse effects of high TBT concentrations (> 100 ng/L) and handling stress from weekly measurements have minimized correlations between growth rates, temperature, and chlorophyll-*a* in Tests 1-4, particularly at the marina sites. In Tests 5-9 growth rate was well correlated with temperature and chlorophyll-*a* concentrations; seasonal effects became more apparent when we switched from weekly to biweekly measurements and seawater TBT concentrations decreased.

We have previously estimated an optimum temperature for juvenile mussel growth in San Diego Bay near 20°C. This is consistent with laboratory predictions (Bayne et al., 1985). In these studies during the coldest winter test, 12-week mean temperatures at all sites were <15°C and growth rates were among the lowest we measured even though mean concentrations of seawater TBT were lower than in most other tests. During the warmest summer test, 12-week mean temperatures ranged from 20.1°C at MSF to 24.4°C at NAV. Growth rates at MSF were the highest ever measured in San Diego Bay during this particular summer test while growth rate at NAV was the lowest of all Navy sites. The highest mean temperature, 25.7°C, was measured in Test 9 at Coronado Cays, the most southern marina site in San Diego Bay. A maximum temperature of 27°C was recorded at this site. Test 9 growth rates at Coronado Cays were lower than at any other marina site except SI. There was no statistically difference in growth rates between mussels transplanted to Coronado Cays and SI. However, seawater TBT concentrations were significantly higher at SI and temperatures were significantly higher at Coronado Cays. Stress from high temperatures near 25°C have been associated with extremely adverse effects on mussels in both the laboratory and the field (Bayne et al., 1985, Wells and Gray, 1960). There are three important factors to consider in the environmental effects of seawater TBT concentration, chlorophyll-*a* and temperature on juvenile mussel growth. They are means, extremes and seasonal variability. The biological significance of the extreme variability has yet to be determined. Laboratory studies have shown that mussels are affected more by continuous than discontinuous exposure to copper (Davenport, 1977) and that they can detect elevated levels of copper and close to avoid exposure (Davenport and Manley, 1978). We do not know how mussels respond to order-of-magnitude shifts in seawater TBT concentrations with tidal cycle in the Shelter Island Yacht Basin previously reported (Clavell et al., 1986) or the weekly variability we measured.

Control over test conditions is the primary advantage of using laboratory toxicity tests for estimating threshold responses to contaminants. Our data emphasize the extreme variability of the nature and lead to questions of how to control and duplicate these conditions in the laboratory. Daily maximum temperature changes of up to 5°C and overall temperature ranges of over 8°C were measured. There were order-of-magnitude shifts in seawater TBT and chlorophyll-*a* concentrations at some sites. Most laboratory studies do not include this

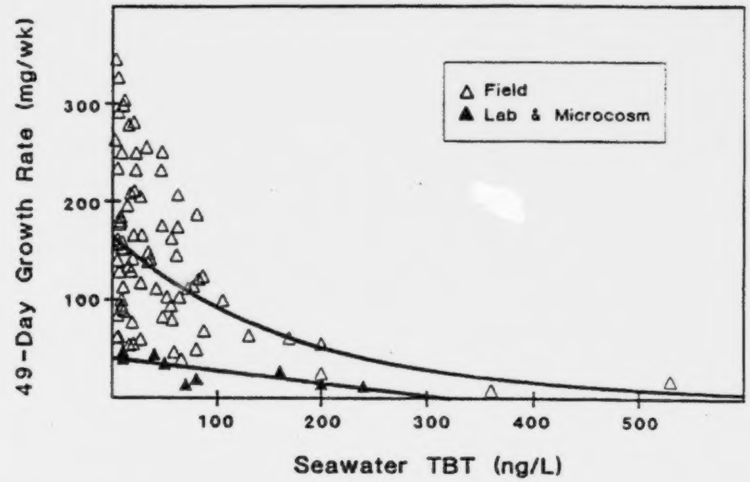


Figure 10. Comparison of 49-day growth rates for juvenile mussels transplanted in the field with juvenile mussel growth rates from laboratory and microcosm studies.

variability or other factors that affect bioavailability of contaminants and mussel growth (Salazar, 1986). It is difficult to determine what laboratory test conditions should be used and how this relates to natural conditions. Figure 10 compares all of the growth measurements we have made in the field with those from our flow-through microcosm work (Salazar and Salazar, 1987) and lab studies in the U.K. using juvenile mussels (Thain, 1986; Thain and Waldock, 1985; Thain and Waldock, 1986). There are order-of-magnitude differences in the results. There are reportedly significant differences when comparing laboratory versus field responses of mussels exposed to TBT in survival, bioaccumulation and growth (Salazar, 1989). It has been shown that maximum mussel growth in nature greatly exceed growth rates measured in the laboratory (Kiorboe et al., 1981).

Monitoring Implications

The importance in making the distinction between using bioindicators to determine the extent of contamination of TBT versus the use of bioindicators to determine environmental effects must be emphasized (Waldock et al., 1990). Chemical monitoring programs that only monitor seawater and tissue concentrations of contaminants document the extent of contamination and not environmental effects. Any assumptions of environmental effects must be inferred from other laboratory or field studies. Biological monitoring programs measure these effects directly. Field measurements provide a realistic test platform for long-term studies, but generally lack the control necessary for experimentation and establishing cause-and-effect relationships, particularly with TBT (Stebbing, 1985). Some control was gained in our experiments by transplanting mussels instead of monitoring natural populations. We were able to identify site-specific differences that could not be determined with natural populations. Pertinent questions that arise are, 1) what to measure, 2) where to measure, and 3) how often to measure it.

What to Measure

The first question to be answered is what species should be used as the biological indicator. We chose mussels because they are found in San Diego Bay, and they have been used in monitoring programs throughout the world. Other biomonitoring programs in San Diego Bay by California Mussel Watch use the Japanese Oyster *Crassostrea gigas* because they are reportedly more sensitive to TBT than mussels and the shell thickening response is reportedly specific to TBT (Stephenson et al., 1986; Smith et al., 1987; Stephenson et al., 1988). We feel this approach is flawed primarily because this species is not naturally found in San Diego Bay nor are they cultured here. It is neither a representative endemic species or of commercial importance in San Diego Bay. An added benefit of mussels is that they can be used to evaluate a variety of contaminants. Additionally, many of the pitfalls have been thoroughly documented and accounted for (Phillips, 1980). This has not been done for shell thickening in oysters. A more generalized approach considers the environment as a whole and separates the effects attributable to TBT with frequent multiple measurements as we have done.

If it is decided to use mussels as the bioindicator of choice, where to get the mussels and what size to use to accurately assess environmental stress becomes an issue. Juveniles were used in our studies to avoid the effects of gametogenesis on growth and because we felt they were more sensitive than adults. We minimized the range of the smallest size mussel (10-12 mm) for practicable collection and to avoid the effects of gametogenesis on growth (Rodhouse et al., 1986) and minimize variability (Phillips and Segar, 1986). However, growth rate was so rapid in some tests that animals were producing gametes by the end of the test. We have also shown a transplant effect and a size effect on bioaccumulation of TBT by mussels (Salazar and Salazar, 1990a). This also has significant monitoring applications. Fischer (1983, 1988) has suggested that bioaccumulation in short-term tests with fast-growing juvenile mussels more accurately reflects recent environmental changes. An integrated monitoring program should include both juveniles and adults.

It is important to use animals from the area that is being studied. Initial mussel studies by Mussel Watch in San Diego Bay included transplants from another site in northern California (Stephenson et al., 1986). Mussel Watch frequently transplants mussels from this pristine site to identify hot-spots of contamination throughout the state by using a standard size and mussel population for a baseline. These comparisons are reasonable and informative. However, if one is attempting to make comparisons within San Diego Bay, it should be demonstrated that the surrogate population is responding like the natural endemic population. Most of the juvenile mussels died in the first San Diego Bay transplant studies by California Mussel Watch, reportedly due to transportation shock. Since these northern California mussels came from a different genetic and environmental stock, it is also possible that they are more sensitive to San Diego Bay contaminants, temperatures and other natural factors. In 67 of the 79 transplants made in our San Diego Bay studies, survival was between 89-100%. Recent electrophoretic studies have even suggested a different species of *Mytilus* in northern California (McDonald and Koehn, 1988). This potential variability must be minimized in monitoring studies.

We have attempted to detect differences between sites using growth to estimate effects and bioaccumulation to document the extent of contamination. There are apparent difficulties in using bioaccumulation in mussel tissues to distinguish differences between particular sites. This may be attributable to the way seawater TBT and tissue TBT are measured. The hydride derivatization method may not truly reflect bioavailable seawater TBT (Salazar, 1986), measured tissue TBT could be biologically inactive (Salazar and Salazar, 1990a), and wet weights of TBT in mussel tissue may obscure true tissue levels (Page and Widdows, 1990). There are unusual problems in attempting to compare wet weight, dry weight and radioactivity-based concentration units (Kelly et al., 1990). It may also be important to consider lipid normalized values since TBT is relatively lipophilic. However, in a recent study with DDT and PCB's, lipid normalized and non-normalized values were not much different in oysters (Sericano et al., 1990).

Where to Measure

The individual site data demonstrate the difficulty in generalizing TBT effects in an area like San Diego Bay or perhaps any estuary, and even within the Shelter Island Yacht Basin. One or two sites with extreme values can easily bias a regional mean and subsequent statistical analyses. Similar problems with extremely high values strongly biasing means in large environmental data sets have been reported from NOAA Status and Trends Programs with bivalve tissues (Uhler et al., 1989; Sericano et al., 1990). A generalized approach often de-emphasizes the variability and extreme values that could be driving biological responses. Identifying these extremes in short-term and long-term variability on a site-specific basis is essential to any monitoring program. To adequately monitor the Shelter Island Yacht Basin, all of the measurements we have made over the last 3 years would have to be concentrated in that area.

The differences in tissue levels of TBT found in our study and those reported by Valkirs et al. (1990) could be attributable to several factors. These include differences between juveniles and adults, sampling frequency, site-specific differences and perhaps even subtle differences in methodology. The natural populations sampled in their monitoring studies were generally limited to the shoreline whereas all water samples were taken in the middle of yacht basins, Navy berthing areas, etc. The primary advantage of transplants is to place animals where they would not normally settle or occur in sufficient quantities to monitor and collect water samples immediately next to the transplant. Without juvenile mussel transplants at the SID bottom site, we would not have been able to show the differences in seawater TBT concentration, tissue TBT concentration and mussel growth rates because mussels are not naturally found there.

How Often to Measure

Most monitoring programs with quarterly sampling plans, like the Navy's statutory monitoring program, are designed primarily to establish long-term trends as well as establishing an environmental baseline. A potential problem with quarterly sampling is that the biological effects may actually be occurring on a weekly scale as in our growth rate measurements or even a daily or hourly scale. Environmentally relevant data from monitoring programs must quantify temporal and spatial variability in contaminants, natural factors and biological responses (Carpenter and Huggett, 1984; Phillips and Segar, 1986). Further, this monitoring must be quantified on an equivalent time scale. Real-time or very short-term chemical and biological measurements are available. Real-time chemical sensors for heavy metals and TBT have been developed in our laboratory and have been used to document the large fluctuations with tidal cycle (Zirino et al., 1978; Clavell et al., 1986). Near real-time cellular, physiological and biochemical measures of biological stress have also been developed (Bayne et al., 1985). We used intensive sampling of seawater, tissues and mussel growth rates to quantify both the extent of TBT contamination and its environmental effects and establish short-term effects. Long-term trends were established by conducting serial seasonal transplant studies over a 3-year period. Our data could be improved significantly with the addition of real-time sampling that more accurately reflected temporal and spatial variability that might affect juvenile mussel growth.

SUMMARY

Serial seasonal transplants of juvenile mussels with frequent growth measurements and chemical sampling have documented temporal and spatial variability in TBT and its effects on growth, bioaccumulation and survival that have not previously been reported. Establishing this variability and identifying site-specific factors affecting juvenile mussel growth in San Diego Bay have facilitated environmentally relevant predictions of threshold levels for TBT effects on mussel growth. This represents the initial calibration of the mussel bioindicator for TBT effects and establishes a significant refinement in the use of mussels as biological indicators. Based on the data presented here, questions have been raised regarding the environmental significance of chemical and biological monitoring programs that may be applicable to all bioindicator responses and monitoring programs. Crucial questions regarding environmental fate and effects of TBT remain unanswered. Sampling must document temporal and spatial variability of the contaminant in question, other contaminants, natural factors that affect biological responses (particularly the bioindicator being used) as well as temporal and spatial variability in the natural biological response. This generic issue of defining an environmentally relevant monitoring program is much more important than the specific issue of TBT effects. All monitoring programs should include chemical measurements to document the extent of contamination and biological indicators to estimate environmental effects.

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AN APPLICATION OF "REAL-TIME" MONITORING IN DECISION MAKING: THE NEW BEDFORD HARBOR PILOT DREDGING PROJECT

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ABSTRACT

A decision-making framework was established for assessing the impacts of a pilot dredging study at the New Bedford Harbor, MA, Superfund site. Concern over possible environmental impacts due to dredging at this site necessitated that a monitoring program be implemented to ensure that unacceptable water quality impacts did not occur during this project. Consequently, criteria were derived, a management committee assembled, and a "real-time" monitoring plan designed. Because many existing chemical concentrations in the water column and indigenous biota exceeded Federal and state water quality limits, site-specific chemical and biological criteria were established. A committee of environmental managers from Federal and state government was established with the authority to assess and modify the operation on a daily basis. Finally, a "real-time" monitoring plan was implemented in which water samples were collected, analyzed within 16 hours, and the data supplied to the management committee in order to assess the environmental impact of the previous days' operation. The combined use of site-specific criteria and a "real-time" decision making management process allowed for successful completion of this project with a minimal effect on water quality.

EXTENDED SUMMARY

New Bedford Harbor (NBH) is located along Buzzards Bay between the cities of New Bedford and Fairhaven, MA. Since the 1940's, electronics and manufacturing companies in the area have discharged effluents containing polychlorinated biphenyls (PCBs) into the harbor. High PCB concentrations in harbor sediments were first documented in 1974 (Connelly and St. John, 1988), with PCB concentrations as high as 100,000 parts per million (ppm) in some areas of the upper harbor. In 1982, the site was added to the Environmental Protection Agency's (EPA) National Priorities List of hazardous waste sites slated for cleanup under the Superfund Act.

A feasibility study conducted by EPA in 1984 proposed several alternatives for the remediation of NBH. One option common to most remediation alternatives was the dredging of contaminated sediments. Federal, state, and local officials, as well as the public, expressed concern that the resuspension of sediments during dredging may cause the release of contaminants that would affect biota at more distant areas in the harbor and Buzzards Bay. Others cited potential pollution problems from contaminated water (leachate) leaking from the proposed disposal site (Averett and Francigues, 1988).

In order to address these concerns, EPA Region I, in conjunction with the U.S. Army Corps of Engineers (COE), initiated the NBH Pilot Dredging Project to establish the impacts of various dredging and disposal options on a small scale with relatively low (with respect to NBH) contaminated sediments (PCB concentrations approximately 100 ppm). Information derived from this project would be used to determine the most environmentally safe methods for use in a possible large scale remediation of the most contaminated areas of the NBH Superfund Site.

The overall goal of the Pilot Project was to determine the feasibility of various dredging and disposal options for removing and sequestering highly contaminated sediments in NBH. This included assessing whether or not it was practical from an engineering perspective, as well as determining if the operations could be completed without causing unacceptable environmental impacts. The engineering aspect of the project assessed three shallow-water dredges capable of removing sediment with minimal resuspension. In addition, two disposal methods were evaluated: 1) a confined disposal facility (CDF), which required construction of a containment dike partially in-water and partially on land; and 2) a confined aquatic disposal cell (CAD), an in situ underwater disposal method (Otis, 1987). The results of these engineering operations are reported elsewhere (Otis, 1989).

A second objective implicit in the overall goal was to determine whether the engineering operations could be completed in such a manner as not to cause unacceptable damage to the environment. The decision-making process used to assess the environmental acceptability of this project is the topic of this summary.

Because of the high PCB concentrations in the sediments to be dredged during the Pilot Project (100 ppm), it was necessary to make rapid assessments as to the environmental "acceptability" of the operations. The evaluation of possible unacceptable contamination of the water column due to dredging during the Pilot Project was complicated by the fact that Federal and State water quality standards for PCBs (U.S. EPA, 1980) and certain heavy metals (U.S. EPA, 1985) were exceeded in NBH under preoperational baseline conditions. In addition, the U.S. Food and Drug Administration (FDA) action level for PCBs in seafood in NBH was exceeded (Kolek and Ceurvels, 1981).

These special conditions necessitated the development of a distinctive site-specific monitoring/management strategy for the Pilot Project. This framework included several unique aspects: 1) development of a set of site-specific Decision Criteria for assessing water and tissue chemical concentrations and biological effects, 2) establishment of a panel of environmental managers, Decision Criteria Committee, to use those data in a timely manner, and 3) design and implementation of a monitoring program to provide the necessary environmental data to the Committee in a rapid time-frame (12-24 hours). This approach provided an effective feedback loop to evaluate, modify or terminate the dredging operation if the Decision Criteria were exceeded.

Each aspect of this strategy was successfully implemented. First, site-specific Decision Criteria were established at two strategic locations within the harbor. The philosophy adopted for establishing criteria values was that short-term, near-field elevations in contaminant concentrations or biological effects would be evaluated against long-term improvements in water quality, provided that no far-field effects were observed. Using this rationale, criteria were established for a number of physical, chemical, and biological parameters based on data collected prior to the initiation of dredging (Nelson and Hansen, In press).

Secondly, a Decision Criteria Committee was formed with representatives from each of the major participants involved in the study: EPA Region I, the COE, the Massachusetts Department of Environmental Protection, and EPA's Environmental Research Laboratory, Narragansett, RI (ERL-N). This committee was empowered to make decisions on a daily basis if there were impacts attributable to the operation. Possible corrective actions to limit adverse effects due to the project ranged from altering operational procedures to temporarily halting the operation or termination of the study.

Finally, a monitoring plan was developed and implemented by ERL-N to collect samples during the operational phases of the project, complete sample analysis within 24 hours, and transmit the resultant information to the Committee for comparison with the Decision Criteria values.

The chemical and biological monitoring data indicated that the dredging operation had a minimal effect on existing water quality; the only criterion exceeded was PCB water concentration. On the four occasions when elevated PCB concentrations were detected, they were attributed to a specific causative operational procedure or meteorological event. Operational modifications were implemented effectively, thus limiting elevations in water column PCB concentrations.

It may be unrealistic to expect to complete a Superfund remediation at an aquatic site with absolutely zero short-term impact. However, this program successfully established a set of limits (Decision Criteria) beyond which the impact was considered unacceptable, and a mechanism (real-time monitoring program) which provided the information necessary for environmental managers (Decision Criteria Committee) to effectively oversee this project to completion.

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EPA'S ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM: AN ECOLOGICAL STATUS AND TRENDS PROGRAM

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ABSTRACT

The U.S. Environmental Protection Agency has initiated the Environmental Monitoring and Assessment Program (EMAP) to monitor the status and trends of the nation's near coastal waters, forests, wetlands, agroecosystems, surface waters, and arid lands. The program is also intended to evaluate the effectiveness of Agency policies at protecting ecological resources occurring in these systems. Monitoring data collected for all these natural resources will be integrated for national status and trends assessments. The near coastal component of EMAP consists of estuaries, coastal waters, coastal and estuarine wetlands, and Great Lakes. The country's near coastal resources have been regionalized and classified, an integrated sampling strategy has been developed, and quality assurance/quality control procedures and data management designs have been implemented. EPA and NOAA have agreed to coordinate and, to the extent possible, integrate near coastal component of EMAP with NOAA National Status and Trends Program. A demonstration project was jointly conducted in the estuaries of the mid-Atlantic states (Chesapeake Bay to Cape Cod) in the summer of 1990. In 1991, monitoring will continue in the mid-Atlantic estuaries and will be initiated in estuaries in part of the Gulf of Mexico.

INTRODUCTION

Environmental regulatory programs have been estimated to cost more than $70 billion annually, yet the means to assess their effect on the environment over the long term do not exist. While regulatory programs are based upon our best understanding of the environment at the time of their development, it is critical that long-term monitoring programs be in place to confirm the effectiveness of these programs in achieving the environmental goals and to corroborate the science upon which they are based.

The U.S. Environmental Protection Agency (EPA), the U.S. Congress, and private environmental organizations have long recognized the need to improve our ability to document the condition of our environment. Congressional hearings in 1984 on the Monitoring Improvement act concluded that, despite considerable expenditures on monitoring, federal agencies could assess neither the status of ecological resources nor the overall progress toward legally-mandated goals of mitigating or preventing adverse ecological effects. In the last decade, articles and editorials in professional journals of the environmental sciences have repeatedly called for the collection of more relevant and comparable ecological data and easy access to those data for the research community. The most commonly suggested tools for accomplishing these goals include a national ecological survey and a bureau of environmental statistics.

Affirming the existence of a major gap in our environmental data and recognizing the broad base of support for better environmental monitoring, the EPA Science Advisory Board recommended that EPA initiate a program that would both monitor ecological status and trends, as well as to develop innovative methods for anticipating emerging problems before they reach crisis proportions (SAB 1988). EPA was encouraged to become more active in ecological monitoring because its regulatory responsibilities require quantitative, scientific assessments of the complex effects of anthropogenic activities on ecosystems. The Environmental Monitoring and Assessment Program (EMAP) is EPA's response to all of these recommendations.

EPA's Office of Research and Development, in concert with several other federal agencies, is developing EMAP to monitor the condition of ecological resources to ensure that our environmental protection efforts are achieving the desired results. When fully implemented, EMAP will be able to respond to the following questions:

- What proportion of the nation's ecological resources are degrading or improving, where, and at what rate?

- What are the likely causes of the observed degraded conditions?

- What is the current status, extent, and geographic distribution of our ecological resources?

- Are control and mitigation programs effective in maintaining or improving the quality of our resources?

OBJECTIVES OF EMAP

To provide the information necessary to address the above questions and the goal of the program, EMAP has established the three following objectives:

1. To estimate the current status, extent, changes, and trends in indicators of the condition of the nation's ecological resources on a regional basis with known confidence,

2. To monitor indicators of pollutant exposure and habitat condition and seek associations between man-induced stresses and ecological conditions,

and

3. To provide periodic statistical summaries and interpretative reports on ecological status and trends to the EPA Administrator and the public.

APPROACH AND RATIONALE

Assessing the status and trends for the nation's ecological resources requires data collected in a standardized manner, over large geographic scales, for long periods of time. Such assessments cannot be accomplished by aggregating data from the many individual, short-term monitoring programs that have been conducted in the past and are being conducted currently. Differences in the parameters measured, the collection methods used, timing of sample collection, and program objectives severely limit the value of historical monitoring data for conducting regional assessments (Wolfe et al. 1987; NRC 1989, 1990a, 1990b).

EMAP proposes to monitor a defined set of parameters (i.e., indicators of environmental quality) on a regional scale, over a period of decades, using standardized sampling methods with a probability-based sampling design. These characteristics distinguish EMAP from other monitoring programs and provide the data for preparing the regional and national scale assessment that are needed to address the environmental issues of the 1990s and beyond (Reilly 1989; Thomas 1988a, 1988b).

Local programs that measure the same parameters and sample in a manner compatible with EMAP will be able to use EMAP products to obtain a regional and national perspective with which to evaluate the seriousness of local problems. This will assist them in two ways: (1) by determining whether local problems are unique and (2) by facilitating detection of problems that are more easily measured on regional or national scales (e.g., determination of whether apparent declines in values resources are associated with regional changes in climate or is more likely attributable to regional or local changes in pollutant loadings.)

NEAR COASTAL COMPONENT OF EMAP

The near coastal component of EMAP has established its inland boundary as the limit of tidal influence. The outer boundary is the continental shelf break. Ecosystems occurring between these boundaries that ultimately will be sampled are the following:

estuarine and coastal wetlands - submerged lands characterized by periodic or constant saturation and the presence of vegetation adapted to or tolerant of saturated soils.

estuaries - semi-enclosed bodies of water that have a free connection with the open sea and an inflow of freshwater that mixes with the seawater.

coastal waters - waters lying over the continental shelf.

Great Lakes - large freshwater bodies not affected by marine currents; each lake has a unique, complex circulation pattern.

At the present time, EMAP does not have the financial resources to implement regional monitoring programs in all near coastal ecosystems simultaneously. Therefore, a phased implementation is proposed that focuses much of the initial efforts on estuaries (USEPA 1990).

SAMPLING DESIGN

The sampling design for the near coastal component of EMAP has three elements:

- A regionalization scheme for partitioning near coastal resources into regions with similar ecological properties that constitute reasonable reporting units.

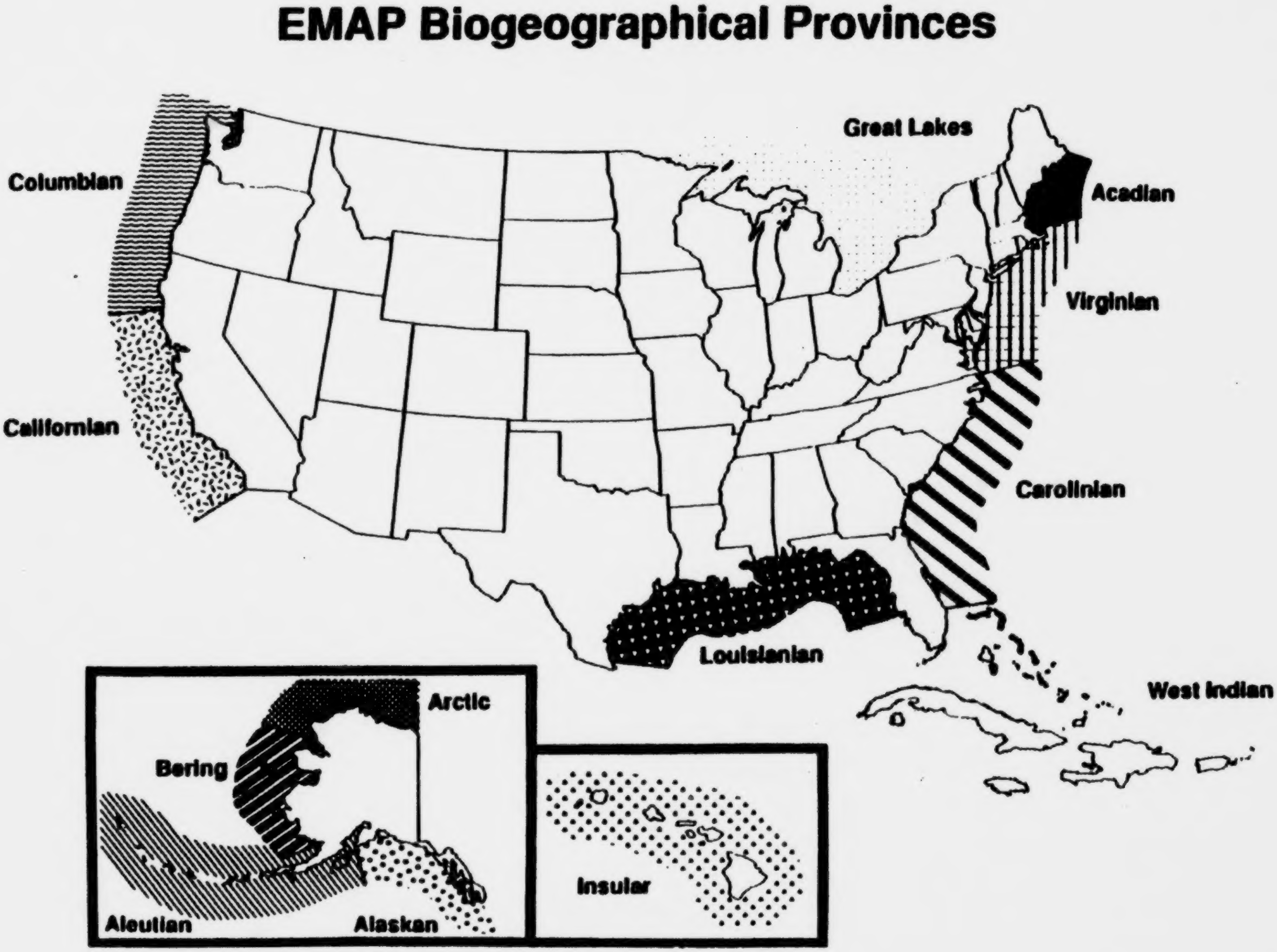
- A classification scheme to define subpopulations of interest (e.g., classes of estuaries, types of wetlands) that can be sampled using a common approach.

- A statistical design that will obtain unbiased estimates of the status and trends of near coastal ecological resources cost effectively.

The regionalization scheme is used to divide the nation's near coastal resources into a series of biogeographical provinces (Figure 1). Initially, the field activities will be implemented in the Virginian Province, with other provinces added in subsequent years. By 1995, all provinces in the continental U.S. should be included in the sampling program.

A classification scheme is used to subdivide estuaries within a province into classes that have similar physical features and are likely to respond to stressors in a similar manner. The classes that are defined include (1) large, continuously distributed estuaries (e.g., Chesapeake Bay, Long Island Sound), (2) large tidal rivers (e.g., Potomac, Delaware Rivers), and (3) small, discretely distributed estuaries, bays, inlets, and tidal creeks and rivers (e.g., Barnegat Bay, Elizabeth River). The purposes of classifying estuaries into categories having similar attributes (e.g., size, shape, resource distributions) are (1) a common sampling design can be applied to each class, (2) the variability in condition within a class should be less that which occurs among classes, reducing the number of samples necessary to characterize a class accurately, and (3) the degree of confidence with which inferences can be made about systems within a class that are not sampled is increased.

A critical issue that must be addressed is how best to represent the ecological condition of near coastal environments with limited financial resources and relatively few samples. It is obvious that one or two samples, from one or two locations, at one time of the day, in a specific season of a particular year cannot characterize the ecological condition of even a small estuary. Such a sampling program is justified only it can be demonstrated that parameters that are indicative of the overall ecological condition can be identified and a population approach to sampling can be used to characterize resources. That is, resources and locations that are sampled can be used to make inferences about unsampled resources and locations. One of the goals of 1990 EMAP-Near Coastal field effort is to make this demonstration.



EMAP Biogeographical Provinces

Columbian
Californian
Great Lakes
Acadian
Virginian
Carolinian
Louisianian
West Indian
Arctic
Bering
Aleutian
Alaskan
Insular

Figure 1. EMAP-Near Coastal regionalization scheme based on biogeographical provinces.

EMAP-Near Coastal does not have the resources to characterize natural variability or to assess status in all seasons. Therefore, sampling will be limited to a confined portion of the year (i.e., an index period), when measured parameters are expected to show the greatest response to pollution stress and within-season variability is expected to be reduced. EMAP-Near Coastal has selected summer as the appropriate index period. For most near coastal ecosystems in the northern hemisphere, mid-summer (July-August) is a period when dissolved oxygen concentrations are most likely to approach stressful low values (Holland et al. 1977; Officer et al. 1984), and the cycling and adverse effects of contaminant exposure are maximal because of low dilution flows and high temperatures (Connell and Miller 1984; Sprague 1985). In addition, fauna and flora are usually abundant during summer, increasing the probability of collecting the organisms required to complete assessments.

Within each estuarine class, elements of systematic, random, and fixed location sampling are used. Large, continuously distributed estuaries are sampled using a randomly placed systematic grid. Grid points are about 18 km apart, and the entire estuary is sampled. Large tidal rivers are sampled along systematically spaced lateral transects. Transects are located about 25 km apart. The starting point for the first transect at the mouth of the river (between river kilometer 0-25) are randomly selected. Two sampling points are located on each transect; one is randomly selected and one is an index sample. The goal of the index sample is to use scientific judgement to identify sampling locations that can be used to determine if degraded conditions occur in a system without having to conduct intensive surveys. The index sample site will be located in a depositional, muddy environment where sediments are accumulating, and the potential for exposure to low dissolved oxygen concentration and/or to contaminants is high. Small, relatively discrete estuaries will be sampled using a population approach. First, a list of all small estuaries is defined and placed in order according to latitude. Then the estuaries are classified into groups of four and one estuary from each group is randomly selected for sampling without replacement. Two sampling points are located in each small estuary that is sampled: one is randomly selected and one is an index sample. Regional scale information from index sites will be combined with similar information from randomly selected locations.

Index samples will be used to estimate the proportion of sampling sites in small estuaries and tidal river segments that have unacceptable (or acceptable) indicator values in places that are particularly vulnerable to pollution impacts. However, the index samples are biased and cannot be used alone to estimate the extent of degradation. When regional scale information from index sites is combined with similar information from randomly selected locations, robust statements can be made about the proportion of systems that have pollution problems in highly vulnerable sites as well as about the extent and magnitude (i.e., areal extent) of degradation for the population of small estuaries and tidal river segments.

When fully implemented, EMAP-Near Coastal will operate on a four-year sampling cycle, with approximately one fourth of the total number of samples needed to make an overall assessment collected in each year. Regional interpretative assessments will be prepared every four years by combining the data collected over the four-year cycle. Such a multi-year baseline reduces the confounding effect of year-related phenomena (e.g., weather) to the assessment process. Multi-year baseline are particularly important for establishing the effectiveness of management actions (USEPA 1983a, 1983b). Annual assessments can be made with the data collected during that year; however, these annual assessment will have a higher degree of uncertainty than assessments based on the full four-year sampling program.

INDICATORS OF ENVIRONMENTAL QUALITY

Many studies have defined the major problems facing the nation's estuaries and near coastal waters (e.g., OTA 1987; USEPA 1983a; NRC 1989; NOAA 1988). In general, these studies conclude that the major environmental issues for near coastal ecosystems are those that adversely affect the maintenance of balanced indigenous populations of fish, shellfish, and other biota including the following:

- Increases in the amount of water that has low dissolved oxygen concentration levels,
- Eutrophication,
- Chemical and microbial contamination of water, sediments, and biological tissue,
- Habitat modification, and
- Cumulative impacts of more that one of the above.

The EMAP-Near Coastal indicator strategy was developed to address these problems and their associated impacts on valued ecosystem attributes.

EMAP-Near Coastal does not have the resources to monitor all ecological parameters of concern to the public, Congress, scientists, and decision makers. Therefore, a defined set of parameters that serve as indicators of environmental quality will be measured. Indicators will be selected to be:

- Related to ecological condition in a way that can be quantified and interpreted,
- Applicable across a range of habitats and biogeographic provinces,
- Valued by and of concern to society, and
- Quantifiable in a standardized manner with a high degree of repeatability.

The selection of indicators that will be used by EMAP-Near Coastal is an ongoing process. It is anticipated that a number of years will be required to develop a complete list of indicators. The selection process consists of the following steps:

- Identification of values ecosystem attributes and stressors that affect them,

- Development of a conceptual source-receptor model that links valued ecosystem attributes to stressors,

- Using the conceptual model to identify candidate indicators,

- Evaluation and classification of candidate indicators into categories (core, development, research) using evaluation criteria that are generic to all EMAP resources(e.g., forest, arid land, agroecosystems),

- Testing and evaluation of indicators to assess their ability to discriminate between polluted and unpolluted sites,

- Conducting regional scale demonstration projects to show the feasibility of indicators and the value of indicator data to characterizing overall ecosystem status, and

- Periodic reevaluation of indicators.

Categories of indicators that will be identified and sampled by EMAP-Near Coastal include the following:

Response indicators - Measurements that quantify the integrated response of ecological resources to individual or multiple stressors. Examples include measures of the condition of individuals (e.g., frequency of tumors or other pathological disorders in fish), populations (e.g., abundance, biomass), and communities (e.g., species composition, diversity).

Exposure indicators - Physical, chemical, and biological measurements that quantify pollutant exposure, habitat degradation, or other causes of degraded ecological condition. Examples include contaminant concentrations in the water, sediment, and biological tissues; the acute toxicity of sediments to endemic or sensitive biota; and dissolved oxygen concentrations.

Habitat indicators - Physical, chemical, and biological measurements that provide basic information about environmental setting. Examples include water depth, salinity, sediment characteristics, and temperature. Habitat indicators will be used to normalize values for exposure and response indicators across environmental gradients. Habitat indicators will also be used as a basis for defining subpopulations of interest for assessments.

Stressor indicators - Economic, social, or engineering measures that can be used to identify sources of pollution and poor ecological condition. Examples include human demographics, land use patterns, discharge records from sewage treatment facilities, freshwater inflows, and pesticide usage on the watershed. Stressor data will be gathered primarily from existing federal and state programs (e.g., NOAA's National Coastal Pollution Discharge Inventory (NCPDI), wetland acreage and extent from USFWS National Wetland Inventory), from other EMAP task groups (e.g., extent and distribution of forests, atmospheric deposition of pollutants), and from local permitting/planning agencies. EMAP-Near Coastal recognizes that it also will have to spend some of its resources on measuring stressors.

The relationships among indicator categories are summarized in Figure 2. Information on exposure, habitat, and stressor indicators will be used to identify potential factors contributing to the status and trends of response indicators. A list of indicators that were used in the first year of the program is provided in Table 1. In the first year, EMAP-Near Coastal is over-sampling indicators and use the data collected to develop a reduced list of indicators that can be applied to characterize overall estuarine condition accurately when the program is fully implemented. The over-sampling is necessary because indicators of estuarine condition that are acceptable to the public and scientists and have been demonstrated to be appropriate to apply at regional scales are not well developed.

ANALYSIS AND INTEGRATION

Integration and synthesis of EMAP-Near Coastal data into assessments of the condition of estuaries is a formidable challenge. Assessment results must be scientifically defensible and presented in a manner that can be understood by non-technical audiences. Unfortunately, estuarine science has not developed measures of environmental condition of estuaries that are accepted by scientists and understood by the public and other non-technical audiences.

To accomplish its objectives, EMAP-Near Coastal will conduct the following types of analyses:

- Status assessments,

- Trends evaluations, and

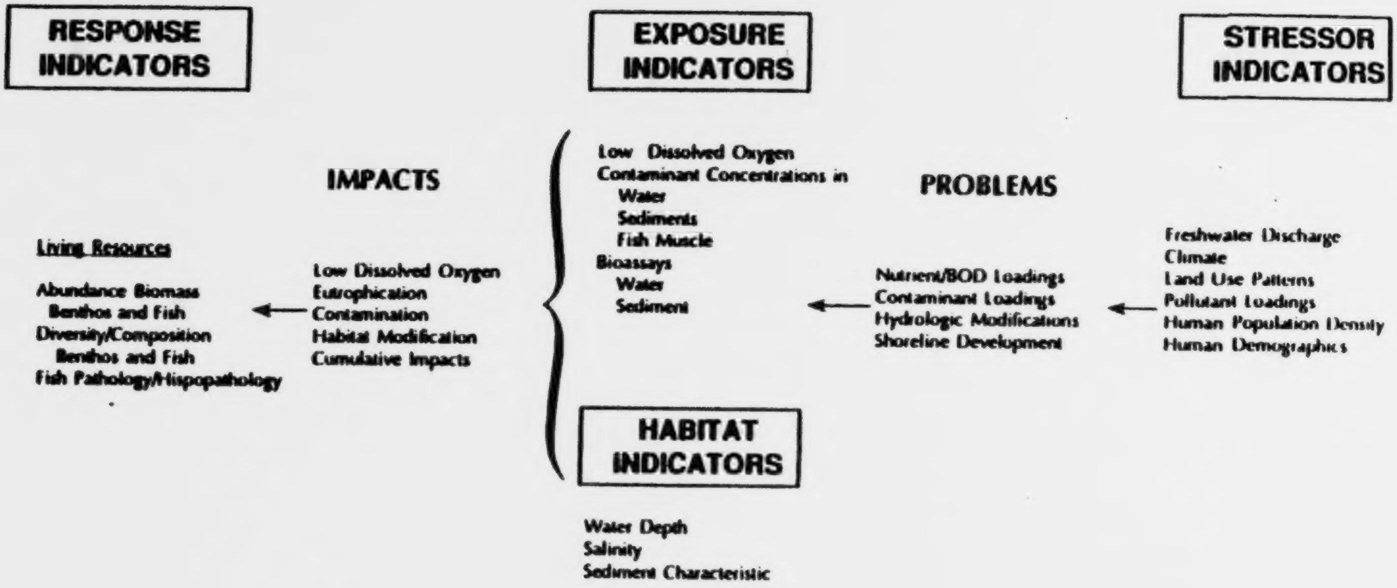
- Diagnostic evaluations including identification of factors that may be affecting status and trends.

The analysis approach for status assessments will be hierarchial. First the overall condition of estuarine resources will be quantified using response indicators to define the extent and magnitude of pollution problems. Then, this integrated assessment will be decomposed to define associations between exposure, habitat, and stressor indicators and to identify likely causes and relative contributions of various stresses to problems.

A principal graphical representation of EMAP status information will be cumulative distribution functions (CDFs). CDFs were chosen because essential information on both central tendency (e.g., mean, median) and extreme values can be summarized in an easily interpreted graphical format (Figures 3). CDFs will be prepared for response indicators for each estuary class, for all estuaries within the region, and eventually for all estuaries nationally. CDFs also will be prepared for selected exposure indicators and to characterize habitat conditions using habitat indicators.

The approach to trend assessment will consist of sampling a portion (e.g., one fourth) of the sampling sites each year in a manner that ensures geographic dispersion and repeating the cycle on a regular basis (e.g., every 4 years). Annual estimates of status can be evaluated individually or aggregated with other years to establish multi-year baseline that are more

EMAP-NC INDICATOR STRATEGY



RESPONSE INDICATORS

EXPOSURE INDICATORS

STRESSOR INDICATORS

IMPACTS

PROBLEMS

Living Resources

Abundance Biomass
  Benthos and Fish
Diversity/Composition
  Benthos and Fish
Fish Pathology/Hispopathology

Low Dissolved Oxygen
Eutrophication
Contamination
Habitat Modification
Cumulative Impacts

Low Dissolved Oxygen
Contaminant Concentrations in
  Water
  Sediments
  Fish Muscle
Bioassays
  Water
  Sediment

Nutrient/BOD Loadings
Contaminant Loadings
Hydrologic Modifications
Shoreline Development

Freshwater Discharge
Climate
Land Use Patterns
Pollutant Loadings
Human Population Density
Human Demographics

HABITAT INDICATORS

Water Depth
Salinity
Sediment Characteristic

Figure 2. Overview of indicator strategy for EMAP-Near Coastal.

EMAP-NC INDICATORS BY MAJOR CATEGORY

Category	Proposed Indicators
Response	Benthic species composition and biomass
	Gross pathology of fish
	Dissolved oxygen concentration
	Fish community composition
	Relative abundance of large burrowing shellfish
	Histopathology of fish
	Apparent RPD depth
Exposure	Sediment contaminant concentration
	Sediment toxicity
	Contaminants in fish flesh
	Contaminants in large bivalves
	Water column toxicity
	Continuous DO measurements
	Contaminant screening
Habitat	Salinity
	Sediment characteristics
	Water depth
Stressor	Fresh water discharge
	Climatic fluctuations
	Pollutant loadings by major category
	Land use patterns of watershed by major categories
	Human population density/demographics

Table 1. List of EMAP-Near Coastal indicators by major category. The manner in which indicators are related to the major environmental problems and impacts is also shown.

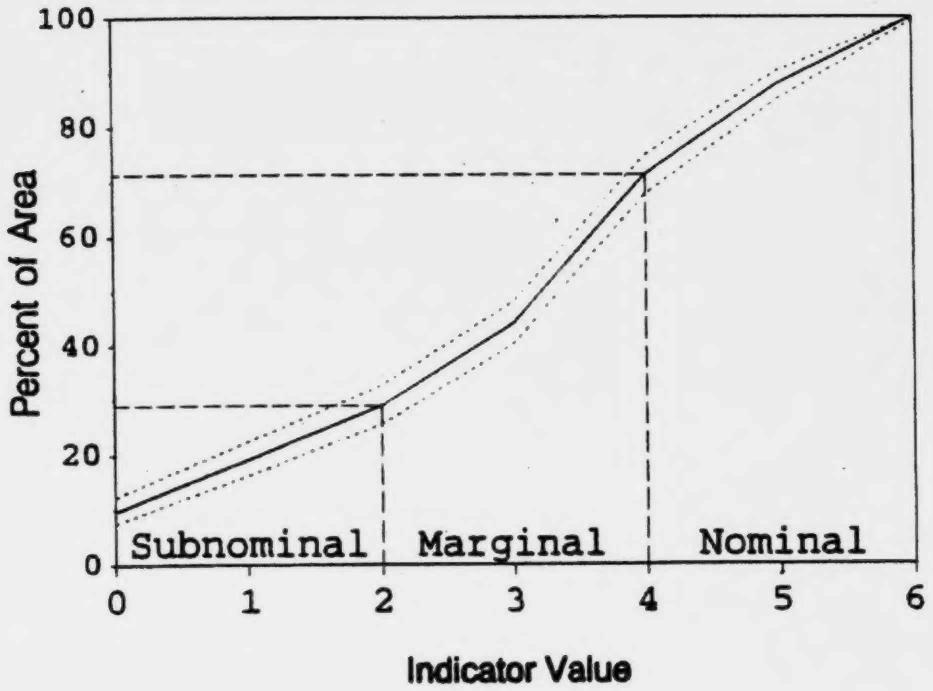


Figure 3. Example cumulative distribution function (CDF). Dotted lines represent confidence limits.

stable than annual estimates. Multi-year baseline are particularly useful for measuring trends and for evaluating the effectiveness of pollution control programs.

Although individual response indicators are important measures of specific aspects of environmental condition, the goal of EMAP-Near Coastal is to provide answers to questions with an holistic perspective of estuarine systems. Multiple statements (i.e., multiple CDFs) about the status and trends of the nation's estuaries, each based on a different response indicator, present information that may confuse many EMAP clients. Single, integrated statements about the overall status of estuarine resources are more easily communicated and understood. Therefore, EMAP-Near Coastal must develop an Estuarine Condition Index (ECI) that integrates the data collected for multiple response indicators into a single CDF describing the status of estuarine resources.

DATA MANAGEMENT

EMAP-Near Coastal will use a distributed data management system. In this system, data are produced at a number of remote locations, where samples are processed. Results are then transferred to a central site where they are verified to be reasonable and are integrated into the Near Coastal Information Management System (NCIMS). The NCIMS will include data in both raw and summary form to minimize costly redundant analysis (NRC 1990a). Information on study characteristics, institutional and organizational structures, sampling methods, sample status, data format, quality assurance, key scientists involved in the generation of each data set, and data access support will be available for all data sets. Data management reports that summarize the types, volume, and quality of data, as well as a list of specific data sets that are available, will be prepared and distributed to potential users frequently (i.e., approximately every two years). For additional information on EMAP-Near Coastal data management, readers should refer to the EMAP-Near Coastal Data Management System Plan (Rosen et al. 1990).

QUALITY ASSURANCE

EMAP-Near Coastal will employ EPA's data quality objective (DQO) approach to ensure that the type, amount, and quality of data collected are adequate to meet program goals and that analysis results have quantifiable and acceptable levels of uncertainty. The DQO process is an iterative approach, balancing costs against uncertainty, to achieve a desired or acceptable level of data quality (Figure 4). The first step in the DQO process consists of determining the level of uncertainty that the decision makers, who will use the data, are willing to accept. Then, the uncertainty associated with the measurement program is estimated. The two estimates are compared and the sampling program modified (e.g., intensity of sampling increased or decreased, sampling methods altered) until the proper balance between costs and uncertainty is achieved. Once an acceptable level of uncertainty has been established, quality control and quality assessment procedures are applied to each program element (e.g., field sampling, laboratory analysis, transfer of information to a data base, and data analysis) to ensure that the specified level of quality is attained and maintained.

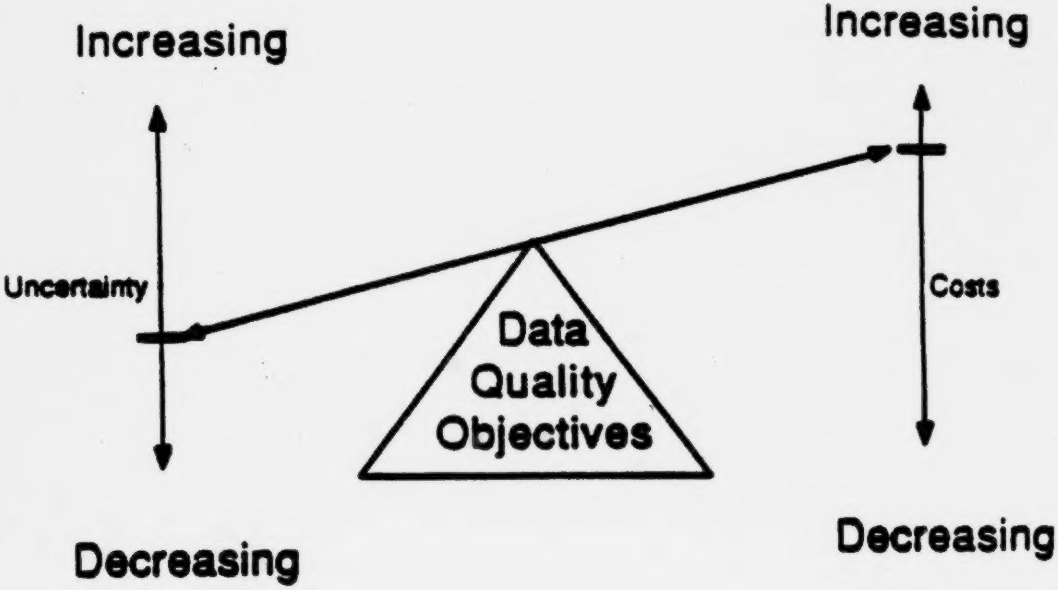

Increasing
Uncertainty
Decreasing
Data Quality Objectives
Increasing
Costs
Decreasing

Figure 4. Role of data quality objectives in obtaining balance between available resources and level of uncertainty.

Because regional data with which to estimate spatial and temporal variability within the summer index period are either unavailable or unaccessible for most, if not all, of the proposed indicators, it will not be possible for EMAP-Near Coastal to implement DQOs during the first year or two of the program. Accordingly, the first year's program will be implemented using measurement quality objectives (MQOs). MQOs establish the acceptable level of uncertainty for field and laboratory methods. MQOs differ from DQOs in that they do not consider spatial and temporal variability in estimating uncertainty levels. The MQO uncertainty level for each indicator is based on the available scientific literature for sampling, processing, and measurement methods or a manufacturer's specification for a given instrument (Plumb 1981; Home and McIntyre 1984; SeaBird Electronics, Inc. 1987; Pollard et al. 1990).

The data collected using MQOs during the first several years of EMAP-Near Coastal will be used to measure the uncertainty associated with the regional measurement program for each indicator. This information will then be evaluated, acceptable DQOs defined, and the sampling program modified as necessary to address program objectives. For additional information on the EMAP-Near Coastal quality assurance program, readers should refer to the Quality Assurance Project Plan developed for the 1990 Demonstration Project in estuaries of the Virginian Province (Pollard et al. 1990). EMAP-Near Coastal also has developed field collection and laboratory processing methods manuals that standardize sampling and processing operations for this study (Strobel 1990; Graves 1990).

1990 DEMONSTRATION PROJECT

As a first step in accomplishing the objectives of EMAP-Near Coastal, a Demonstration Project was implemented in the estuaries of the Virginian Province in 1990. The major goals of this Demonstration Project are to evaluate the utility of the EMAP-sampling design and approach and, at the same time, collect information necessary to develop a technically sound and cost-effective sampling program that can implemented over the long term. The specific goals of the 1990 Virginian Province Demonstration Project include the following:

- Demonstrate the value of regional monitoring data collected in a standard way, measuring a defined set of parameters, using a robust sampling design as a basis for status assessment,

- Identify, test, and evaluate indicators of environmental quality for estuaries that can be applied over broad regions,

- Develop standardized sampling and processing methods for evaluating estuarine environmental quality,

- Evaluate alternative sampling designs and approaches for establishing a regional and national monitoring network in estuaries,

- Develop analysis procedures for converting monitoring data into information useful for the public, Congress, environmental decision makers, policy analysts, and the scientific community, and

- Identify and resolve logistical problems associated with conducting a regional/national scale monitoring program in estuaries.

EMAP-Near Coastal is being implemented in the Virginian Province because there is a general public perception that estuaries in this area are rapidly deteriorating. Additionally, many of the estuaries in this region have been intensively investigated by scientists, and a considerable amount of information has been available for use in designing the Demonstration Project. Finally, many management decisions for this region are forthcoming, including development of a restoration plan for the New York Harbor complex and development and evaluation of management plans of many large estuaries, including Delaware Bay, Chesapeake Bay, and Long Island Sound. Development of these plans presents an opportunity to demonstrate how EMAP information can assist in the formulation of environmental programs and policies.

The 1990 Virginian Province Demonstration Project includes a number of program enhancements. These special studies include:

- An indicator testing and evaluation program that will evaluate the ability of indicators to discriminate between polluted and unpolluted environments,

- Temporal sampling for some indicators (e.g., dissolved oxygen concentration) extending beyond the boundaries of the anticipated index period to better define starting and ending times for the index period,

- Repeated measurements of selected indicators (e.g., dissolved oxygen concentration, fish community characteristics, and contaminants in fish flesh) during the index period to assess their stability and suitability for application in the sampling design, and

- Intensive spatial sampling conducted at a subset of stations (i.e., Delaware River, Delaware Bay, Indian River Estuary) to evaluate the advantages and disadvantages of sampling at alterative spatial scales.

The reporting associated with the 1990 Virginian Province Demonstration Project includes the following:

- A Near Coastal Program Plan for 1990: Estuaries (USEPA 1990), which describes the detailed program plans for the Demonstration Project.

- An Example Assessment Report (Frithsen 1990), that presents examples of the kinds of assessment information that EMAP-Near Coastal will produce.

- A Demonstration Project Activities Summary, due in the winter 1990, that summarizes the data collected, describes the status of data records, identifies and discusses problems and issues encountered during the field program, and develops recommendations for improving logistical activities during the implementation phase.

- A Demonstration Project Assessment Report, due in the summer 1991, that makes a status assessment for the Virginian Province based on one year of data and presents the findings of the indicator testing and evaluation program, intensive spatial sampling efforts, and the evaluation of alternative sampling designs. The report will provide the technical basis for the design of future EMAP-Near Coastal monitoring efforts in the Virginian Province and elsewhere.

COORDINATION

Meeting the objectives of EMAP requires close coordination among many offices within EPA and with other federal, state, and local agencies involved in monitoring activities. Although EMAP is funded by the Office of Research and Development, other offices within EPA (e.g., Office of Marine and Estuarine Protection) have participated actively in its development. EMAP-Near Coastal has coordinated with each of the National Estuary Programs in the Virginian Province about activities in 1990 and beyond. Coordination will occur with other National Estuary Programs and ongoing EPA programs (e.g., Gulf of Mexico Program) before monitoring activities are implemented in these regions.

Both NOAA and EPA have mandates to conduct a broad range of research and monitoring activities to assess the effects of pollution on coastal and estuarine environments. There are similarities and differences between existing NOAA and EPA programs; however, the combined results of both agencies' programs serve the national interest more than the results of individual programs. It is the intention of NOAA and EPA to cooperate and coordinate, to the greatest extent possible, to integrate estuarine and coastal monitoring, research, and assessment activities and to ensure that data collected by EMAP-Near Coastal and NOAA National Status and Trends Program augment and complement each other to the maximum extent possible.

The framework for cooperating and coordinating monitoring and research activities between NOAA and EPA is the NOAA/EPA Committee for Coastal and Estuarine Environmental Quality Monitoring. This committee was created to ensure coordination and exchange of information between the two agencies on coastal monitoring, research, and assessment. The joint committee has held monthly meetings since October 1989. The purpose of these meetings has been to exchange planning information and to identify opportunities for joint complementary activities. As a result of the activities of this committee, a joint NOAA/EPA quality assurance program has bee implemented for sampling near coastal environments. Through the joint committee, NOAA has assisted EPA in the development and evaluation of coastal and estuarine environmental quality indicators by participating in workshops, providing data for retrospective analyses, and reviewing EPA plans and analysis results. The joint NOAA/E(A committee recently developed and executed a Memorandum of Understanding (MOU) that defines continued interagency cooperation and interaction and provides a framework for integrating activities of NOAA National Status and Trends Program and EMAP-Near Coastal into a unified national monitoring and assessment program for estuarine and coastal waters (see Appendix B in USEPA 1990).

Coordination with NOAA and other federal agencies, as well as with other offices within EPA, avoids duplicative monitoring efforts and allows existing data to be used to maximum benefit. This coordination should lead to the incorporation of historical baselines established by other agencies, such as NOAA's baselines on contaminant concentrations in sediments and bivalves, into EMAP-Near Coastal analyses. It will also lead to the incorporation of EMAP-Near Coastal data into the analyses and assessments accomplished by other agencies. The regional-scale assessments resulting from EMAP-Near Coastal, in combination with the ongoing characterization work of NOAA, will provide a substantial portion of the technical information needed to (1) characterize existing conditions and define coastal environmental problems; (2) coordinate the design and implementation of regional monitoring and assessment activities; and (3) identify, assess, and recommend management strategies and solutions that will enhance and protect regional coastal environmental quality.

FUTURE FIELD ACTIVITY PLANS

In 1991, estuarine sampling will continue in the Virginian Province, and a Demonstration Project will begin in the estuaries of the Louisianian Province. Progress reports on these activities will be available in 1992. During the summer of 1992, EMAP-Near Coastal expects to begin sampling estuaries in the Carolinian Province.

ACKNOWLEDGEMENTS

Although this work was supported by the U.S. Environmental Protection Agency, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency; no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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PLATFORM SESSION

Toxicology & Regulation I & II

Chairs: R. Scroggins & J. Sprague

BIOLOGICAL TESTING DEVELOPMENT IN ENVIRONMENT CANADA.

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ABSTRACT

For some years there has been a quiet struggle to enhance the acceptance, usage and regulatory application of ecotoxicological testing and data. This was deemed necessary to effectively implement and enforce the Canadian Environmental Protection Act and Fisheries Act, and to deliver other existing and emerging environmental quality legislation, policy and programmes (ie: Pest Control Products Act, Transportation of Dangerous Goods Amendments, MARPOL). Various actions were recommended or initiatives taken to address our biological testing program deficiencies. These have met with varying degrees of success.

INTRODUCTION

What are biological tests? For our purpose, and discussion here, we confine our scope of interest to biological tests directly applicable to environmental protection. Such tests measure the response of living organisms which have been exposed to chemical, physical or biological contaminants. The results of these tests can be used to detect, assess and control the effects of those contaminating (toxic) substances. The types of biological tests required by Environment Canada programs are those used to estimate the ecotoxicity (toxicity, genotoxicity, pathogenicity, persistence, bioaccumulation etc.) of toxic substances such as may be released into the environment (eg. chemicals, effluents, leachates) or already found in the receiving environment (eg. soils, sediments, water).

What is the mandate for biological testing and data use? Biological testing and associated data are essential for the delivery of departmental legislation, policies and programs. The Canadian Environmental Protection Act (CEPA), and its proposed regulations (eg. New Chemical Pre-Notification), contain mandatory requirements and other stipulations for using biological tests. The general provisions of the Fisheries Act and its regulations use toxicity as a parameter for controlling effluents and for prosecuting offenses involving the release of deleterious substances. Biological test data are necessary to support priority issues of the Environmental Agenda (eg. marine environmental quality) and the operating philosophies and strategies of the Department (eg. sustainable development). Conservation and Protection (C&P) cradle-to-grave approach to toxic substance management, requires support by activities which procure and apply toxic effects data. The assessment of pesticide use hazards (via PCPA) relies on ecotoxicological support data. Legislative amendments to control the transport of dangerous goods in Canada (via TDGA) are planned and will likely specify biological testing requirements for hazardous waste classification. In addition, Environment Canada has many international obligations involving ecotoxicity under agreements with OECD, MARPOL, the London Dumping Convention and the UNEP 1985 Montreal Guidelines.

THE PROBLEM

In recent years, increasing concern has been expressed over the lack of development of biological testing within Environment Canada. Inconsistencies existed with respect to the amount, manner and effectiveness of biological test use. Managers have historically been uncertain with respect to the role of biological testing in C&P programs and what level of testing capability should be developed. Overall, past efforts in the biological testing area have been fragmented and have had little national focus within Canada. It is felt by many that Canada has failed to keep pace with other agencies and countries with respect to the implementation of biological testing in pollution control strategies and policies. For example, the US-EPA has integrated acute and chronic toxicity testing requirements into their national wastewater abatement program. Perhaps the most pressing concern was that C&P had not seriously started to develop the biological testing tools, framework, QA program, guidance on sample collection and preparation, which would be required to meet new operational and program needs.

REMEDIAL ACTIVITIES

In 1986, the Technology Development Branch (Environmental Protection) supported a study to examine the above concerns and recommend corrective measures. The study involved representatives from all Regions and HQ Branches of C&P. A second major source of advice was from the federal-provincial Intergovernmental Aquatic Toxicity Group (IGATG). A report (Sergy 1987) was subsequently submitted to the C&P Laboratory Managers Committee (LMC). The report provided conclusions on the various issues and recommended curative actions related to the current problem, the application of and requirement for biological testing, and in-house testing capability. It recommended a battery of tests best fitted to meet C&P program needs, and proposed a plan to put those tests on-line. This included a strategy for developing national protocols and procedures.

In spring of 1988 a C&P workshop was held at Alliston, Ontario, on the role of biology in the new regulatory framework. Participants representing government, industry, consulting and university sectors provided valuable feedback to Environment Canada. The workshop proceedings (Day et al 1988) identified deficiencies in the current use of biological tests in Canada and lead to specific recommended actions for Environment Canada to consider.

In 1989, the recommendations of Sergy (1987), Day (1988), and the advice of IGATG and the LMC, were packaged into a 'program development plan'. Specific needs and actions were identified to address biological testing deficiencies in C&P. Resource requirements were estimated for a five year period. The actions were grouped into five broad areas of need, being;

- Develop and adopt a policy statement and program framework for the use of biological tests and procedures in environmental protection and the setting of biology-based environmental control standards.
- Conduct the applied research and developmental work required for the preparation of standardized biological test methods and approaches which are needed for delivery of C&P environmental legislation and programs.
- Provide national guidance and mechanisms to enhance the quality and uniformity of biological test data collected and produced by public and private sector laboratories (QA/QC).
- Establish and maintain an efficient in-house 'national' capability for biological testing required for the identification and assessment of toxic substance hazards and for regulatory program auditing and enforcement requirements.
- Enhance communications and technology transfer to promote the effective use and understanding of biological testing by the public, scientists, regulators and industry.

The program development plan has been presented in many forums to various levels of Environment Canada management and used in funding submissions.

During these latter activities, basic principles or policy on the use of biological tests have also been advocated, namely, that C&P

> *-support the effective utilization of integrated biological, chemical and physical techniques and approaches.* The limitations of approaches which are too singular or narrow in scope and the value of complementary biological test data are well recognized.

-apply biology-based environmental quality and protection standards, where they are appropriate. National standards (regulations, guidelines, objectives, operational codes, etc.) should reflect desirable levels of environmental quality and environmentally sound operational practices. Biological tests and test data will have a role in the derivation and description of such standards.

-apply a range of functional biological tests and procedures, which measure lethal, sublethal, short and long-term effects, and account for the varied sensitivities of different trophic levels and species. No single biological test can, by itself, be expected to satisfy the varied needs in environmental protection.

Of course none of these activities have been conducted in isolation, and other agencies in Canada have been advancing the improvement of biological testing. A good example is the Ontario MISA program which will require acute lethality testing and the control of all direct discharges of effluent to the environment.

OUR REPORT CARD

The various activities have met with some degree of success, albeit limited in many areas.

- In C&P aquatic toxicity laboratories some new testing capabilities have been brought on-line and staff training in new techniques increased.
- The federal/provincial IGATG committee has grown in membership and scope of representation. It has become much more vital and has taken an active role in the provision of advice especially in the area of new test method development.
- There have been improvements to the toxicity provisions of the Fisheries Act Regulations.
- In management circles, the level of awareness about toxicity testing and its value has definitely increased. Nevertheless, overall funding for biotesting initiatives remains at a minimal level and there is still much room for improvement.

A great success has been achieved on one front, that of developing Canadianized-standardized-regulatory-biological testing procedures. I believe that we are now producing some of the best procedural documents in the world. Action on this one element commenced in 1987 and we are now in our third year. Interest and support from C&P management has in fact grown. Our progress in this regard is discussed by McLeay et al (1990).

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STATUS OF DEVELOPMENT, ENVIRONMENT CANADA BIOLOGICAL TEST METHODS

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ABSTRACT

Commencing in 1988, a program was initiated to develop standardized biological test methods appropriate for Environment Canada's needs regarding aquatic environmental monitoring and regulation. Standard tests for determining the acute lethality of chemicals, effluents, elutriates, leachates or receiving waters to rainbow trout (Oncorhynchus mykiss), threespine stickleback (Gasterosteus aculeatus) and Daphnia spp. have been written and published at the instigation of Environment Canada, under guidance of Canada's federal/provincial Inter-Governmental Aquatic Toxicity Group (IGATG). Reference methods for determining the acute lethality of effluents to rainbow trout and Daphnia magna have also been prepared and published.

Environment Canada methodology documents presently undergoing internal or external review prior to publication include: a rapid toxicity test using the luminescent bacterium Photobacterium phosphoreum; a microplate test for growth inhibition using the green alga Selenastrum capricornutum; a test for reproduction and survival using the cladoceran Ceriodaphnia dubia; and a test for larval growth and survival using the fathead minnow, Pimephales promelas. Procedures undergoing final development or initial drafting are: a sediment lethality test using marine or estuarine infaunal amphipods; an in-vitro test for genotoxicity; and a test for fertilization success and embryo survival using echinoids. Additional procedures have been identified by IGATG as a general priority for standardization.

INTRODUCTION

During the mid-1980s, certain individuals within Environment Canada recognized the need to develop and apply a suite of biological test methods for evaluating and monitoring test materials destined for or within the Canadian aquatic environment. In consultation with the federal/provincial Canadian Inter-Governmental Aquatic Toxicity Group[1] (IGATG), a set of biological test methods appropriate to freshwater, estuarine or marine environments was proposed for development and standardization (Sergy 1987). Using this set of recommended test methods as a guideline, a multi-year program was initiated in 1988 to develop standardized biological test methods appropriate to meet Environment Canada's varied needs in environmental protection (Sergy and Scroggins 1990).

The process towards methodology development and standardization now in effect requires the active participation of all IGATG members, under the direction of Environment Canada. It also requires input and review from the research and technical staff within Environment Canada's biological testing laboratories. Experimental verification of various test procedures and conditions under consideration is frequently required throughout the developmental process, which may take two or more years per document.

Priorities for document development are appraised and revised during bi-annual IGATG meetings. A document-specific subcommittee, comprised of members of IGATG as well as other informed government, private or university researchers, is struck to review initial drafts. Work on an individual method approved for development is then commenced by compiling and reviewing similar procedural documents available internationally or within Canada. Related publications, technical reports and unpublished laboratory findings are also reviewed at this stage. Following draft preparation and internal revision by the authors, the document is thoroughly reviewed by the subcommittee members and written comments provided the authors regarding necessary changes. Special meetings with IGATG and/or subcommittee members may be required to resolve contentious issues. A final draft is then prepared and distributed for external review by informed parties within Environment Canada, Fisheries & Oceans Canada, provincial environmental ministries, U.S. practitioners, and the private sector involved with biological testing. IGATG and subcommittee members also participate again at this stage of review and revision. Written comments received from the external review are considered by the authors and Environment Canada's scientific authorities, and a final manuscript prepared for publication.

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[1] Established in the mid-1970s, this group represents the technical heads of the federal and provincial aquatic toxicity laboratories across Canada. The scope of IGATG is applied aquatic toxicology in the service of federal and provincial environmental agencies, with a special emphasis on industrial effluent control.

Following is a status report regarding biological test methods developed to date by Environment Canada, as well as those presently being prepared or considered for future development. The current status of this test-method developmental program is outlined in Table 1. Some of the more important test conditions and procedures for certain of the test methods are summarized here to provide initial familiarity with the test. The reader is directed to available methodology documents for complete information.

GUIDANCE DOCUMENT FOR USING REFERENCE TOXICANTS

A report entitled "Guidance Document on Control of Toxicity Test Precision Using Reference Toxicants" has been prepared (Environment Canada 1990a). This document describes the appropriate uses of reference toxicants as part of the procedure for conducting standardized biological tests. Recommendations are made regarding the choice of reference toxicants for specific test methods. Information is provided regarding the acquisition and handling of reference chemicals. Guidance for testing frequency and chemical confirmation of test solutions is given. Recommended procedures for establishing and updating warning charts, and for the interpretation of data derived from biological tests with reference toxicants, are also given in this document.

ACUTE LETHALITY TESTS USING RAINBOW TROUT, THREESPINE STICKLEBACK, OR DAPHNIDS

The development of separate procedural documents for undertaking acute lethality tests using rainbow trout (Oncorhynchus mykiss; formerly Salmo gairdneri), threespine stickleback (Gasterosteus aculeatus), or daphnids (Daphnia magna or D. pulex) began during

TABLE 1. Current status of Environment Canada's developmental program for biological test methods.

PROCEDURES IN PRINT

1. Acute Lethality Test Using Rainbow Trout
2. Acute Lethality Test Using Daphnia spp.
3. Acute Lethality Test Using Threespine Stickleback
4. Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout
5. Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*

PROCEDURES UNDERGOING INTERNAL OR EXTERNAL REVIEW

6. Toxicity Test using the Luminescent Bacterium *Photobacterium phosphoreum*
7. Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia*
8. Test of Larval Growth and Survival using Fathead Minnows
9. Test of Growth Inhibition using the Green Alga *Selenastrum capricornutum*

PROCEDURES UNDERGOING FINAL DEVELOPMENT OR INITIAL DRAFTING

10. Sediment Lethality Test using Marine or Estuarine Infaunal Amphipods
11. Test for In-Vitro Genotoxicity
12. Test for Fertilization Success and Embryo Survival using Echinoids

PROCEDURES IDENTIFIED AS A GENERAL PRIORITY FOR STANDARDIZATION

13. Test of Growth Inhibition using Aquatic Macrophytes
14. Test of Growth Inhibition using Multiple Species of Algae
15. Sediment Lethality Test using the Freshwater Amphipod *Hyalella azteca*
16. Acute Lethality Test using Green Algae
17. Acute Lethality Test using *Artemia* sp.
18. Test of Bioaccumulation using *Macoma balthica*
19. Test of Bioconcentration using Fish
20. Biodegradation Test
21. Test of Growth Inhibition using Protozoans

the summer of 1988. Published methods are now available (Environment Canada 1990b,c,d).

The format for each of these three test methods is the same. General or universal conditions and procedures are outlined for undertaking an acute lethality test using a variety of test materials. Additional conditions and procedures are stipulated which are specific for assessing samples of chemicals, effluents, elutriates, leachates, or receiving waters. Included are instructions on holding and acclimating test organisms, sample handling and storage, test facility requirements, procedures for preparing test solutions and test initiation, specified test conditions, appropriate observations and measurements, endpoints, methods of calculation, and the use of reference toxicants.

Test with Rainbow Trout

The acute lethality test using rainbow trout (Environment Canada 1990b) is to be undertaken as a static assay (i.e. test solutions are not renewed during the test). If the toxicity of effluent, leachate, elutriate or receiving water is being determined using this test, a sample volume of 25 L or more (depending on fish size, concentrations being tested, need for replicates) is normally required. Test duration is for 96 h (4 days).

An uncontaminated supply of fresh water, whether dechlorinated municipal drinking water, surface water, groundwater or reconstituted water, may be used for holding and acclimating fish and as the control/dilution water. Hatchery-reared fish weighing between 0.3 and 5.0 g may be used for this test, provided that they are not diseased and that they have been acclimated to test conditions for a minimum of 2 weeks prior to testing. A minimum of 10 fish is to be exposed to each test solution. Fish-loading density in test solutions must not exceed 0.5 g/L. Fish are not to be fed for 24 h prior to nor during the test. Test temperature is 15 ± 1 C. Test solutions are aerated minimally (≤ 7.5 mL/min·L^{-1}) throughout the test. Lighting, using full-spectrum fluorescent tubes, should provide up to 500 lux at the surface of each test solution, regulated to give a daily photoperiod of 16 ± 1 h of light and 8 ± 1 h of dark.

Phenol and/or zinc are recommended as reference toxicants. Test endpoints may be percentage fish-mortality at 96 h or an estimate of time to 50% mortality (LT50) or, for multiple-concentration tests, a computer-derived median lethal concentration (96-h LC50). The test is rendered invalid if mortality in the control water exceeds 10% or if more than 10% of the control fish appear atypical during the test.

Test with Threespine Stickleback

The acute lethality test using threespine stickleback (Environment Canada 1990c) is also a static 96-h test. Sample-volume requirements are normally 25 - 100 L. Natural or artificial seawater (salinity full-strength or brackish, depending on test objectives) is used for acclimating stickleback to laboratory conditions and as control/dilution water for this test. Test fish may be captured from coastal waters or cultured, and should weigh between 0.2 and 3 g at the time of the test.

As with the acute lethality test using rainbow trout, a minimum of 10 fish are exposed to each test solution, including one or more controls (water only). Fish-loading density should not exceed 0.5 g/L and fish should not be fed 24 h prior to nor during the test. Test temperature is normally 10 ± 2 C. Lighting, aeration conditions, recommended reference toxicants, test endpoints and criteria for test validity are the same as those for the acute lethality test using rainbow trout.

Test with Daphnids

Unless specified otherwise for regulatory or other purposes, either *Daphnia magna* or *Daphnia pulex* may be used when conducting acute lethality tests (Environment Canada 1990d). *D. pulex* may be used at any hardness, and this species is recommended if the hardness of the control/dilution water is less than 80 mg/L. *D. magna* may be used as the test species if the hardness of the control/dilution water is equal to or greater than this value. This test is performed as a static 48-h assay.

Fresh water used for culturing organisms and as the control/dilution water may, depending on test objectives, availability and the nature of the test material, be natural or reconstituted. Neonates $\leq$24 h old are used for the test. At least 10 neonates should be exposed to each test solution; replicate (e.g. triplicate) solutions are frequently used. Density of daphnids in solutions should not exceed one animal per 15 mL. The test should be conducted at 20 ± 2 C. During the test, solutions are not aerated and daphnids are not fed. Lighting conditions using "cool-white" fluorescent illumination are preferred, and should be regulated to provide a daily photoperiod of 16 ± 1 h light and 8 ± 1 h dark, with no more than 800 lux at the surface of test solutions.

If the toxicity of effluent, leachate, elutriate or receiving water is to be evaluated using this test, a sample volume of 2 L is normally adequate. One or more of NaCl, $K^2Cr_2O_7$ or $ZnSO_4$ are recommended for use as reference toxicants for the test. Test endpoints may be percentage mortality at 48 h, a computer-derived LC50, or LT50 for one or more test concentrations. Cultures of daphnids to be used in toxicity tests should meet the health criteria specified in Environment Canada (1990d). The test is not valid if mortality in the control exceeds 10% or if >10% of control organisms show overt, stressed behaviour (e.g., immobility).

TOXICITY TEST USING LUMINESCENT BACTERIA (Photobacterium phosphoreum)

Late in 1988, Environment Canada commenced the review and adaptation of a commercially-available ("Microtox^{TM}") rapid toxicity test using the marine luminescent bacterium Photobacterium phosphoreum. Internal revision and subcommittee review of initial drafts took place during 1989. A final draft has now being submitted for external review. Publication of this document is scheduled for 1991.

The standardized procedure adapted by Environment Canada for undertaking this test is consistent with the Microtox^{TM} toxicity test, which is the exclusive property of Microbics Corp. of Carlsbad, California. Reagents and apparatus required to perform this test are available commercially. The test is now marketed and used around the world, and there is an extensive scientific literature on the test and on the results of using it.

Environment Canada's final-draft document outlines general or universal conditions and procedures for testing a variety of materials using the Microtox toxicity test. Additional conditions and procedures are stipulated which are specific for assessing samples of chemicals, effluents, elutriates, leachates, receiving waters and sediments. Included are instructions on sample handling and storage, test facility requirements, procedures for preparing test solutions and initiating tests, specified test conditions, appropriate observations and measurements, endpoints, methods of calculation, and the use of reference toxicants.

One of the principal advantages of this test is its rapidity. A test can normally be completed within 15 minutes from time of start. Test organisms are marketed in a stable (lyophilized) form, and can be quickly reactivated before testing. The bacteria emit light as a result of normal metabolic processes, and the light is measured with a standard photodetection device under specific conditions. Reduction of light at 5, 15 or 30 minutes is taken as a measure of sample toxicity.

The standard procedure for performing the Microtox toxicity test involves the testing of a series of four serial dilutions of the sample plus control(s). Light outputs of cuvettes of reactivated bacteria are measured before the sample is added, then again after 5 and 15 minutes of exposure, and perhaps after 30 minutes or longer if there are slow-acting toxic agents. The readings are corrected according to the change in the control(s), to allow for drifts in light output over time and small effects from dilution of the bacteria when sample is added. A dose-effect curve is analyzed and the concentration causing 50% inhibition of light production is estimated mathematically.

Although a relatively small sample would be enough to carry out the test, 500-mL or 1-L samples of effluent, elutriate, leachate or receiving water are recommended to allow portions to be used for measuring initial pH and dissolved oxygen concentration. The Microtox test is normally carried out at 2% salinity by adding a salinity-adjusting solution to the sample and using a control/dilution water that is 2% salinity. Samples that are more saline may also be tested if desired, in which case the salinity of the control/dilution water should be adjusted to that of the sample. In any event, it is important to carry out the test at ≥2% salinity. Test temperature is 15 ± 0.2 C. Phenol is recommended as a reference toxicant.

MICROPLATE GROWTH INHIBITION TEST USING Selenastrum capricornutum

The development of a standardized chronic toxicity test which determines inhibition of growth of the freshwater alga Selenastrum capricornutum due to aquatic contaminants, was initiated by Environment Canada during 1989. A published document representing this biological test method is expected to be available in early 1991. Research by Environment Canada scientists which preceded the development of this test method has been published previously (Blaise et al. 1986, Thellen et al. 1989).

The format used in preparing this methodology document is consistent with that evident in Environment Canada's other biological test methods now published and available. Universal procedures and conditions for preparing algal cultures and conducting microplate growth inhibition tests are given. Specific procedures for testing chemicals or samples of effluent, elutriate, leachate or receiving water are provided. Procedures required to test volatile toxicants are also delineated.

A sample volume of only 5 - 10 mL is required to perform the microplate algal toxicity assay, although larger volumes may be necessary for chemical analyses. Test duration has been standardized at 4 days (96 h). Preparation of test solutions and determination of cell counts at test completion can be automated, making it a cost-efficient assay. The initial cell density for the microplate algal growth inhibition test is 20,000 cells/mL. This chronic toxicity test is conducted without aeration at a temperature of 24 ± 2 C. Reference toxicants recommended for use with the test include zinc, potassium dichromate and phenol. The biological endpoint for this algal assay is cell density at 96 h, which can be determined using an electronic particle counter or manually using a microscope and hemocytometer. For multiple-concentration tests, the test endpoint is calculated and expressed as the IC50, i.e. the concentration of sample which causes a 50% reduction in the growth of the algal population.

CHRONIC OR SUB-CHRONIC TOXICITY TESTS USING *Ceriodaphnia dubia* AND FATHEAD MINNOWS

A final-draft document entitled "Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*" was prepared for external distribution and review during June 1990. Publication of this biological test method by Environment Canada is scheduled for 1991. A fourth-draft document "Test of Larval Growth and Survival Using Fathead Minnows" was distributed to subcommittee members for review during August 1990. This test method is also scheduled for publication by Environment Canada during 1991.

These biological test methods have been modified from the research of Donald Mount and Teresa Norberg-King, U.S. Environmental Protection Agency, Duluth, Minnesota, and the resulting U.S. EPA (1989) methods manual for measuring chronic toxicity. Consistent with the approach taken in preparing the biological test methods for determining acute lethality of chemicals, effluents, elutriates, leachates or receiving waters, each of these methods describes culturing procedures and conditions, universal test conditions and procedures, and procedures for determining the toxicity of specific types of test materials. As a member of the respective subcommittees responsible for the primary review of these two procedural documents, Ms. Norberg-King is actively contributing to their evolution.

Test with daphnids

Environment Canada's chronic toxicity test using Ceriodaphnia dubia is being developed as a three-brood, static-renewal toxicity test. Each test solution is to be replaced at intervals of $\leq$24 h throughout the test. The test is continued until at least 60% of the first-generation test organisms in the control solutions have produced three broods. At the recommended test temperature of 25 ± 1 C, this should occur within 7 ± 1 days.

Uncontaminated groundwater, surface water, dechlorinated municipal water or reconstituted fresh water may be used for culturing organisms and as the control/dilution water. The test is initiated using a single neonate ($\leq$24 h old) organism per 15-mL volume of test solution in each of ten replicate test vessels. Daphnids are fed daily throughout the test. Test solutions are not to be aerated. Lighting using "cool white" fluorescent fixtures to provide a daily photoperiod of 16 ± 1h light and 8 ± 1h dark, with intensity $\leq$600 lux at the surface of the solutions, is recommended.

A 2-L volume of sample (e.g. effluent, receiving water) is adequate for most tests. One or more of NaCl, phenol or $ZnSO_4$ are recommended for use as reference toxicants for the test. Biological endpoints are based on the reduction in the number of live neonates produced by each first-generation daphnid during the test period, and on increased mortality of the first-generation daphnids. Using each of these indices of effect, the Lowest-Observed-Effect Concentration (LOEC) and the No-Observed-Effect Concentration (NOEC) may be derived statistically. The Inhibition Concentration percentage (ICp) may also be calculated.

Environment Canada's draft document specifies health criteria that should be met by cultures of C. dubia before their use in toxicity tests. It also states that the test is not valid if mortality of first-generation test organisms in the control water exceed 20% and/or reproduction in the controls averages <15 live young per surviving adult.

Test with Fathead Minnows

The sub-chronic test for growth and survival of larval fathead minnows requires daily replacement of test solutions throughout the 7-day test period. Fresh water, whether uncontaminated ground, surface, reconstituted or, if necessary, dechlorinated municipal water, may be used for culturing fish and as water for preparing the control solutions and all dilutions of test material. Test solutions are normally prepared in triplicate, with 15 larvae per 250 or 500 mL of solution in each test vessel.

Larvae in each test solution should be fed two or three times daily with newly-hatched brine shrimp nauplii.

Test temperature should be in the range of 25 ± 2 C. Test solutions are normally not aerated. The dissolved oxygen content in test vessels should not fall below 40% of saturation unless such an effect is considered part of the test; aeration of all vessels may be required in certain instances to prevent this. Lighting conditions for culturing and testing should be provided using full-spectrum fluorescent fixtures, regulated to give a daily photoperiod of 16 ± 1h light and 8 ± 1h darkness, with an intensity of no more than 500 lux at the water/solution surface. If the toxicity of effluent, elutriate, leachate or receiving water is being determined using this test, a sample volume of 4 L is normally adequate. One or more of $ZnSO_4$, NaCl or phenol are recommended for use as reference toxicants.

There are two biological endpoints for the test, the first being adverse effect on growth of fish as measured by mean dry weight for each group of larvae at the end of the 7-day exposure. The other endpoint is increased mortality. The Lowest-Observed-Effect Concentration (LOEC) and the No-Observed-Effect Concentration (NOEC) may be derived statistically using each of these indices of effect. The Inhibition Concentration percentage (ICp) may also be calculated. The test is considered invalid if mortality in the control water exceeds 20%, or if more than 20% of these fish display atypical swimming or loss of equilibrium. Additionally, control fish should average 250 ug dry weight or greater at the end of the test.

SOLID-PHASE SEDIMENT ASSAY FOR TOXICITY USING MARINE OR ESTUARINE INFAUNAL AMPHIPODS

Environment Canada is presently developing an assay for sediment toxicity using a number of species of marine or estuarine infaunal amphipods found within Canadian coastal waters/sediments. Publication of this biological test method is scheduled for 1991.

The test method will be largely in keeping with the document developed by the American Society for Testing and Materials through the efforts of the task group co-chaired by Janet Lamberson and Richard Swartz of the U.S. Environmental Protection Agency (ASTM 1989). Dr. Swartz is a member of the subcommittee responsible for the primary review of Environment Canada's methodology document.

The formulation of this biological test method is necessitating appreciable evaluation of candidate test species by Environment Canada's laboratories in the Atlantic and Pacific & Yukon Regions. Initial testing with reference toxicants, reference and contaminated sediments and the amphipods *Rhepoxynius abronius* and/or *Corophium volutator* was conducted in 1988 and 1989. An inter-laboratory comparison (government laboratories and EVS Consultants Ltd.) of the relative sensitivity of these and other candidate test organisms collected from Canada's Atlantic or Pacific coasts is scheduled for the fall/winter of 1990. Species of sand-burrowing marine or estuarine amphipods being sought within Canadian coastal waters for this laboratory assessment include the following:

Atlantic Coast	Pacific Coast
Phoxocephalus holbolli	*Rhepoxynius abronius*
Corophium volutator	*Foxiphalus xiximeus*
Ampelisca vadorum	*Eohaustorius estuarius*
Amphiporeia lawrenciana	*Grandifoxus grandis*
Amphiporeia virginiana	*Monoculodes spinipes*
Pontoporeia femorata	

These tests will be undertaken as static 10-day assays (ASTM 1989). Tests with control (from the site where organisms are collected), reference or contaminated sediments are normally performed using 20 individuals per replicate and 5 replicates per treatment. Each replicate consists of a 2-cm layer of test sediment and an overlying layer of uncontaminated seawater. Amphipods are not fed during the test. Test temperature is normally 15 ± 2 C. Seawater in test vessels is aerated minimally throughout the 10-day test period. Cadmium chloride is used to assess the acute lethal tolerance (96-h LC50) of the test organisms to a range of concentrations of this reference toxicant in seawater.

The primary test endpoint is percentage mortality at 10 days. Other endpoints may include a determination and comparison of percentage emergence and/or percentage reburial for each test sediment. In instances where a range of concentrations of the suspect sediment is prepared by mixing with control or other uncontaminated sediment, the test endpoint may be a 10-day LC50.

ADDITIONAL BIOLOGICAL TEST METHODS FOR STANDARDIZATION BY ENVIRONMENT CANADA

During 1989, regional laboratories of Environment Canada began investigating a biological test method using echinoderms (sea urchins, sand dollars). Development of standardized marine assays with these organisms, using fertilization success and embryo survival as biological endpoints, will be started in early 1991. Test procedures and conditions developed by Drs. Paul Dinnel and Quentin Stober at the University of Washington in Seattle (Dinnel and Stober 1985) will be used as a guideline in developing this test method. Their participation as subcommittee members responsible for primary review of draft documents is being sought.

Other biological test methods now being considered by IGATG and Environment Canada as priorities for development and standardization are shown in Table 1. This list should be considered as tentative only, and may be revised in the near future to accommodate changing program needs.

REFERENCE METHODS FOR DETERMINING ACUTE LETHALITY OF EFFLUENTS TO RAINBOW TROUT AND *Daphnia magna*

During 1989, Environment Canada identified a need to develop standardized protocols for determining the acute lethality of effluents to rainbow trout and *Daphnia magna*. These were required in conjunction with legislation associated with the revised (effective 1991) Federal regulations for control of water pollution from pulp and paper mills (Scroggins 1990), and with respect to the monitoring and regulation of other effluent types and sources by Environment Canada . Accordingly, separate reference-method documents which define test conditions and procedures to be used for determining the acute lethality of effluent samples, using rainbow trout and *Daphnia magna* as test organisms, were prepared during 1989/1990. These documents are now published and available (Environment Canada 1990e,f).

Training videos which depict the approved method for culturing test organisms (rainbow trout or *Daphnia magna*) and for conducting the test are presently being prepared by Environment Canada. The intent of the videos is to provide guidance, consistent with the protocol documents, regarding the preparation for and conduct of each biological test method. Copies of these videos will be made available to industry, private consultants and government laboratories as soon as they are completed (scheduled for early 1991).

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BIOLOGICAL TESTING PROVISIONS OF THE NEW FEDERAL PULP AND PAPER EFFLUENT REGULATIONS.

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On July 1, 1991, new national regulations to further restrict liquid effluent discharges of deleterious substances to the environment from Canadian pulp and paper operations will come into force. These regulations have been developed using the authority provided through the Fisheries Act and the Canadian Environmental Protection Act. The biological testing provisions of the new regulations and implications of these requirements for industry, government and environmental consultants will be discussed. Future Environment Canada plans to further restrict pulp and paper mill effluent discharges and the challenge facing environmental toxicologists and ecologists will be covered as well.

REPORT CARD ON THE USE OF TOXICITY TESTS TO CONTROL THE DISCHARGE OF TOXIC EFFLUENTS IN THE UNITED STATES.

T.J. Norberg-King, U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory-Duluth, MN 55804 (218-720-5529).

Over the last ten years in the United States, the use of toxicity tests to control toxic discharges has increased. At first tests were used on acutely toxic effluents, and then methods were developed to determine either the sublethal or chronic toxicity of effluents. These techniques were applied to test effluents in actual discharge sites to determine if laboratory toxicity tests could predict impact on the receiving waters. The availability of these tests resulted in issuance of a policy statement by EPA and a subsequent Technical Support Document was written to implement the policy. This presentation will discuss the status of the use of this approach in the U.S. and the research effort currently underway to address the cause of toxicity in effluents.

FACTORS AFFECTING EFFLUENT TOXICITY TESTING GUIDELINES IN MINNESOTA

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The use of toxicity testing has expanded greatly in recent years due to federal regulatory emphasis. The development of relatively simple and low cost static renewal toxicity tests, particularly the new early life stage chronic tests, has helped make their regulatory use possible. These test methods were accompanied by concurrent development of methodology that combined toxicity testing with techniques which manipulated or fractionated effluent samples to attempt isolation of toxicant characteristics or identification of individual toxicants in effluents. These procedures provided further impetus for treatment remedies. The process of detecting toxicity, identifying toxicants, and assessing control options has been termed a Toxicity Reduction Evaluation or TRE. The emergence of these methodologies has identified two issues having important impacts on those dischargers being regulated with toxicity testing requirements. The first issue is inherent in static testing where it has been observed that effluent pH often increases between solution renewals. The second issue needing clarification is the appropriate frequency of toxic events necessary to the completion of a successful TRE.

Description of Issues

The Minnesota Pollution Control Agency (MPCA) has its own toxicity testing program and has conducted tests on nearly all of the mechanical wastewater treatment plants in Minnesota. State regulations require dischargers to exhibit no acute toxicity at the point of discharge or in the mixing zone. No chronic toxicity is allowed at the edge of the mixing zone. Experience in conducting both flow through and static acute toxicity tests revealed a particular phenomenon which was occurring in the static tests. At the end of a typical 24-hour period between solution renewals, the pH of whole effluent would frequently increase 0.5 pH units or more above initial pH measurements. Proportionately smaller pH increases were observed with more highly diluted effluent solutions. This phenomenon is not observed in flow through acute tests where effluent solutions are continuously dispensed to test chambers. Often accompanying the pH increase in the static tests was an increase in mortality. Such changes in pH can have dramatic effects on toxicants and toxicity, most notably ammonia, which is a common constituent of wastewater from biological treatment systems.

Once toxicity is found, a decision needs to be made regarding when it is appropriate to enter into a TRE. This discussion has become centered on the frequency of toxicity necessary to the completion of a successful TRE. Toxicity which is found infrequently stands little chance of successfully completing a TRE, whereas an effluent that is consistently toxic stands an excellent change of being resolved. Somewhere between these extremes exists a threshold frequency of toxicity below which a TRE would not be required.

Discussion

It is believed the pH changes observed in static tests can be attributed to characteristics of wastewater treatment. Microorganisms responsible for waste treatment require oxygen, respire carbon dioxide and produce ammonia. Carbon dioxide in solution in the wastewater depresses pH. As solids are removed before discharge, the carbon dioxide becomes in excess of equilibrium with the atmosphere. To establish a new equilibrium, carbon dioxide must be lost to the atmosphere, thereby increasing pH. The evolution of carbon dioxide from effluent occurs during static tests from the moment of sampling, through test initiation, to solution renewal 24 hours later. This can be further exacerbated when test protocol temperatures are higher than effluent sample temperatures.

Increase in pH alone does not create the observed toxicity. The presence of ammonia during these pH changes can provide significant toxicity. The effect of pH on ammonia toxicity has been described by Wuhrmann and Woker (1948), Tabata (1962), Thurston et al. (1984), and U.S. EPA (1984). The concentration of un-ionized ammonia, the most toxic form of aqueous ammonia, exists in higher proportions at higher pH. At 25°C the percent of un-ionized ammonia in solution increases nearly tenfold between pH 7 and pH 8 from 0.566% to 5.38% (Thurston et al. 1979). Conversely it has been demonstrated that un-ionized ammonia is less toxic at higher pH until about pH 8 where toxicity remains nearly constant with subsequent increases (U.S.EPA 1984). For warmwater species, the one hour un-ionized ammonia criterion calculated at 25°C and pH 7 is 0.131 mg/l NH3. At pH 8 the criterion is 0.37 mg/l NH3, a nearly threefold difference. It can be seen that the concentration of un-ionized ammonia increases faster with increasing pH than does the decrease in toxicity, providing a net toxicity increase between pH 7 and pH 8. The exposure of organisms in ammonia laden effluents to pH elevations is typically not seen with flow through tests.

Preliminary data being collected on wastewater treatment plants indicate that total ammonia concentrations with well operated systems exhibit definite seasonal fluctuations. Total ammonia concentrations are reduced considerably during the summer months from their winter high levels due to nitrification. Table 1 illustrates examples of a treatment system designed to nitrify during summer (Plant A), a system designed to meet secondary treatment standards without nitrification design requirements (Plant B), an oxygen assisted activated sludge system (Plant C), and a secondary treatment system which is at or above hydraulic or conventional pollutant treatment capacity and is presently under expansion (Plant D). Plants A, B, and D all have elevated total ammonia concentrations during the winter. Plants A and B exhibit the effects of nitrification with Plant A showing a higher nitrification rate (0.9 mg/l total ammonia) due to its design for ammonia removal. Plant D shows the effects of reduced nitrification capability during the summer because of its marginal treatment of conventional pollutants. Plant C total ammonia concentrations remain low consistently, possibly due to the corresponding high rate of treatment afforded conventional pollutants.

Table 1. Mean seasonal total ammonia concentrations (mg/l) for select Minnesota municipal wastewater treatment plants (MWWTP)

	MWWTP			
Season	A	B	C	D
Dec-Mar	13.5	10.7	1.3	18.0
Apr-May	8.6	8.4	2.6	16.8
Jun-Sep	0.9	4.5	1.7	10.9
Oct-Nov	8.4	6.6	1.2	13.0

Further, un-ionized ammonia exhibits a characteristic species sensitivity pattern that aids in acute test interpretation for ammonia toxicity. Un-ionized ammonia is most toxic to fathead minnows (Pimephales promelas), followed in decreasing order by the water fleas, Ceriodaphnia dubia and Daphnia magna.

Implications

The significance of seasonal ammonia fluctuations on the interpretation of toxicity for whole effluent tests is substantial. Effluents discharged during cold weather months when ammonia is elevated will typically be colder than test protocol temperatures and indicate a lower pH. Tests conducted on effluents at this time would likely demonstrate toxicity that would not be present under temperature and pH conditions existing at discharge or during mixing in the receiving water. In addition, any chronic tests conducted at this time would be testing sensitive endpoints which characteristically do not occur for warm water species in that season. Since pH increases during the exposure period in static tests, it would seem appropriate to interpret toxicity test results in light of actual point of discharge conditions or to the projected conditions expected in the mixing zone.

Interpretting the pH/ammonia relationship is important to the toxicity testing program because of the possibility that much of the observed toxicity we have encountered in past tests can be attributed to this relationship. However, once toxicity is determined to be present, whether it be ammonia or some other toxicant, it still remains to be decided when a discharger should begin conducting a TRE. Presently, limited data and TRE experience prevent resolution of the issue at this time.

Effect on Guidelines

To address these issues, the MPCA has produced a set of general guidelines for determining toxicity of effluents and specific criteria for conducting a TRE. These guidelines are based on a finding of valid, prevalent, and unacceptable toxicity. Tests are valid when toxicity is accurately portrayed and false positives or negatives are reasonably minimized. Toxicity can be determined to be prevalent when its magnitude, duration, frequency, and effects on species sensitivity are known. Unacceptable refers to the degree of acute or chronic toxicity as expressed by appropriately measured biological endpoints.

Interpretation of the pH/ammonia relationship is necessary in discerning test validity under the toxicity test guidelines. To limit interpretational problems and be consistent with the guidelines, it has been necessary to rearrange test protocols and test scheduling in discharge permits. Chronic tests are not scheduled for the cold weather months and winter acute tests are conducted at 10°C to minimize pH drift. Data on pH, temperature, and total ammonia are collected at the time of discharge sampling, and at the beginning and end of each test renewal phase. Scheduling changes, chemical behavior of ammonia with pH, species sensitivity patterns to ammonia, and seasonal nitrification patterns of wastewater treatment plants aid the interpretation of toxic conditions at the point of discharge and in the mixing zone.

The magnitude of toxicity is reflected in TRE guideline threshold levels which are based on limited experience with acute serial dilution tests. Most of the toxicity found was less than 3 acute toxicity units (TUa) measured at discharge. Chronic test threshold levels are arbitrarily set at 3 chronic toxicity units (TUc) set at the edge of the mixing zone.

The lack of any comprehensive database relating frequency of toxicity to the success in conducting a TRE prevents accurate definition of a frequency threshold. For TRE guidelines, a 20 percent threshold has been adopted based on a potential for 3 positive test battery results out a maximum 14 test batteries that may be conducted in the life of a discharge permit.

These general toxicity guidelines form the basis for criteria that require a discharger to move from a monitoring phase into a TRE:

1) No TRE is required and monitoring is continued when;
toxicity is found less than 20 percent of the time at low magnitudes (< 3 TUa at discharge or < 3 TUc at the edge of the mixing zone). Toxicity is short in duration (< 24 hours) and is exhibited in one species, but may not reoccur with that same species.

2) Evaluate the need for a TRE when;
toxicity of low magnitude and longer duration is found at frequencies above 20 percent. Species sensitivity to toxicity is inconsistent, but more than one species exhibits toxicity at any one time.

3) A TRE is required when;
toxicity exists at longer durations with frequencies well above 20 percent. The magnitude of toxicity may range from low to high, and the pattern of species sensitivity is consistent.

Conclusion

Toxicity testing and toxicant identification have seen rapid developments in recent years. It is anticipated that future techniques will address the ammonia issue, and a more comprehensive data base will be forthcoming to evaluate the association of frequency of toxicity with successful TRE's. It is possible that present initial phase TRE techniques could be combined at the monitoring stage. However, most commercial laboratories in the region are not capable of this now. The frequency with which toxicity is found in a discharge will determine the probability of success of any requirement to conduct a TRE.

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SUBLETHAL TOXICITY TESTING AT THE EDGE OF THE MIXING ZONE: THE WISCONSIN APPROACH.

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Beginning in 1988, aquatic life toxicity testing was required in several NPDES permits issued by the State of Wisconsin. To evaluate the potential for sublethal effects to fish and aquatic life in receiving waters, certain point source dischargers were required to perform the Fathead Minnow Larval Survival and Growth test and the *Ceriodaphnia dubia* Survival and Reproduction test. Since 1988, several modifications have been made which were conducive to program enhancement. These modifications have been both administrative (e.g., program implementation) as well as methodological (e.g., test protocols). This paper will present a brief program history along with the major program modifications that have occurred and their associated regulatory consequences. Furthermore, this paper will address the significance of requiring sublethal toxicity tests in which the "pass/fail" criterion is based on an assumption of very limited assimilative capacity of the receiving water.

A PERSPECTIVE ON BIOLOGICAL ASSESSMENTS: MONSANTO'S EXPERIENCE

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BACKGROUND

Since issuance of USEPA's national policy for water quality-based toxics control and supporting technical guidance manuals, effluent toxicity testing and toxicity reduction evaluation requirements have rapidly emerged as an important component of many industrial and municipal NPDES permits. Of the approximately 3800 U.S. industrial facilities with NPDES permits, over one-third now contain effluent toxicity testing requirements.

During the past twelve years, Monsanto has been actively involved in performing effluent toxicity tests, developing procedures for assessing effluent safety and negotiating effluent toxicity testing requirements in NPDES permits. The authors have been directly involved in over 50 NPDES permit negotiations and discussions related to whole effluent toxicity testing requirements in Monsanto plant NPDES permits. The purpose of this paper is to highlight some of the important aspects of Monsanto's effluent toxicity testing and assessment experience and its application to EPA's national policy and technical guidance documents. Recommendations will be provided on approaches for further improving EPA's guidance to the States and EPA regions and the effectiveness of biological assessments in the permitting environment.

AQUATIC HAZARD EVALUATION OF EFFLUENTS

Recognizing that available resources and time are often limited and there are large numbers of discharges that need testing, decisions must be made to test effluents only to the point that a confident decision can be reached on aquatic hazard. Tier testing, the collection of data in a step-wise sequential manner, with decisions based upon exposure and effects data, has provided a cost effective and scientifically defensible mechanism for determining the aquatic safety of both chemicals and whole effluents (Kimerle et al., 1986).

When making decisions about the safety of a wastewater, the relationship between exposure and toxics effects data as well as the uncertainty associated with the data base must be clearly understood. Wastewaters exhibiting a large margin of safety between the environmental exposure concentration and the laboratory derived toxicological "safe" concentration, can generally be judged safer than wastewaters exhibiting small or no margin of safety. The water quality criterion concept relies upon the above relationship since recognizes that exposure concentrations should not exceed the calculated water quality criterion.

TEST METHOD PRECISION

During the past decade, there has been considerable debate regarding the ability to reproduce data using whole effluent toxicity test methodologies. In 1983, Monsanto sponsored a nine laboratory round-robin study of EPA's Daphnia magna static, acute effluent toxicity test (Grothe and Kimerle, 1985). The study examined both inter-and intralaboratory variability. The intralaboratory and interlaboratory coefficients of variation were determined to be 22% and 33%, respectively. Similar levels of precision have been observed for other whole effluent toxicity and chemical specific test methods (Rue et al., 1988).

NPDES PERMIT EXPERIENCE

Like many other industries, whole effluent toxicity testing requirements has become an integral part of most Monsanto plant NPDES permits. To date, eleven Monsanto plant sites have effluent toxicity testing requirements in the Special Conditions Section of their NPDES permits. While such testing is now a common requirement in Monsanto plant permits, the specific test conditions proposed and ultimately incorporated into the permits vary considerably among states and EPA regions. Many NPDES permit writers and their permits frequently refer to EPA's water quality-based toxics control policy and technical guidance manuals as the basis for their proposed conditions. However, relatively few agencies appear to be utilizing EPA's guidance on effluent safety assessments as outlined in the Technical Support Document (Grothe et al., 1990).

SPECIFIC CONCERNS RELATING TO EPA'S GUIDANCE ON WHOLE EFFLUENT SAFETY ASSESSMENTS

In general, EPA's effluent toxicity testing guidance in the TSD is technically sound and valuable for assessing wastewater safety and establishing wastewater evaluation priorities. However, some issues in the TSD need additional clarification/modification in order to increase the application of hazard assessment concepts in permits and achieve more consistent application of effluent safety assessments in the NPDES permit program.

In both EPA's water quality based toxics control policy and Technical Support Documents, EPA recognizes the importance of understanding receiving water impact and exposure concentrations in assessing effluent safety. For example, EPA states that where there is a significant likelihood of impact to receiving water biota, toxicity limits and a TRE may be required. EPA goes on to state that toxic impact is characterized by measuring effluent toxicity and comparing to exposure levels. While such guidance is technically sound, a number of regulatory agencies are not establishing effluent safety assessment requirements in NPDES permits but instead are immediately establish pass/fail numerical toxicity limits and TRE requirements. In other words, decisions are being made about the safety of wastewaters without an appropriate toxicological and exposure data base. Potential problems with establishing effluent toxicity limits without a toxicological and exposure data base should be discussed in EPA's guidance document.

Another concern area is EPA's guidance on outfall diffusers. According to EPA, if a discharger does not have a high rate diffuser at the end of its outfall to achieve rapid mixing, the effluent prior to entering the river must not be acutely toxic. However, if a high rate diffuser is present, a discharge could be acutely toxic as long as acutely toxic conditions are confined to a very small area around the diffuser structure. While the use of high rate diffusers makes sense and will be appropriate for some discharge situations, it is erroneous to assume that this strategy should be applied to all discharges.

While no acute toxicity may be necessary for some discharge situations, it is inappropriate to assume that discharges must exhibit no acute toxicity to be environmentally acceptable. It is likewise erroneus to assume that a discharge lacking a high rate diffuser is adversely impacting the aquatic environment. Existing outfall structures, effluent and receiving stream flow rates may already result in relatively rapid mixing of a wastewater in the aquatic environment such that acutely toxic conditions either do not exist in the receiving stream or are limited to a small zone of initial mixing as discussed in the Technical Support Document. To require dischargers to arbitrarily achieve no acute toxicity at the end-of-the-pipe or install high rate diffusers will lead us back down the path of treatment for treatment sake and the resulting unnecessary and costly outfall modifications. The goal of the water quality based approach is to protect aquatic life in the receiving body of water, not in the discharge pipe. By utilizing the concepts of aquatic hazard assessment discussed earlier, the toxicity of a wastewater can be evaluated in light of predicted or measured exposure levels to ensure that appropriate decisions are made regarding the need for corrective action.

RECOMMENDATIONS FOR IMPROVING IMPLEMENTATION OF THE WATER QUALITY-BASED APPROACH AND TOXICITY TESTS IN THE NPDES PERMIT PROGRAM

There are a number of things that can be done to help clarify EPA's technical guidance and improve the implementation of the water quality-based approach and whole effluent toxicity testing in the NPDES permit program. Implementation of these recommendations should ensure that more consistent, scientifically defensible approaches are utilized to evaluate effluent safety. They also will help promote more wide scale acceptance of effluent toxicity testing within the NPDES permit program.

1. The use of aquatic safety assessments prior to setting numerical toxicity limits should be clearly promoted in regulatory programs.

2. An aquatic hazard assessment and successive periods of non-compliance, not a single test result, should be used as the basis for a TRE.

3. Dischargers should be allowed to demonstrate that existing structures, effluent, and mixing characteristics already meet EPA's instream acute and chronic toxicity criteria. Toxicity tests conducted on water samples collected from the receiving stream in the vicinity of the plant outfall would be useful for determine if receiving stream toxicity criteria are being achieved. Dye-dilution studies could also be used to more accurately quantify near-field and far-field mixing and dilution.

4. Additional guidance needs to be provided to permit writers concerning appropriate criteria for initiation of a TRE. Each state and EPA region should adopt a clear definition of a TRE so that permitees can clearly understand what is expected when such requirements are proposed in permits.

5. EPA should continue to sponsor workshops to educate the regulatory and regulated communities on effluent toxicity assessment and TRE procedures.

6. Round-robin studies need to be performed on effluent toxicity test methods so that questions regarding the precision of specific test methods can be resolved.

7. Studies should continue to examine the correlation between laboratory and field results to better understand the reliability of predicting field impacts from laboratory tests.

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APPLICATION OF MARINE BIOLOGICAL TESTS IN ASSESSING AND CONTROLLING THE ACUTE AND CHRONIC TOXICITIES OF EFFLUENTS *

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INTRODUCTION

Coastal areas worldwide are being increasingly degraded or destroyed, largely due to industrial and municipal waste-water discharges. Approximately half the world's population lives within 100 miles of the coast; this is predicted to increase. Much of the industry is there also.

Most environmental scientists and regulators understand the usefulness of aquatic toxicity tests for assessing and controlling liquid effluents discharged to the environment. However, in Canada, the use of marine toxicity tests is not well developed nor widely used. Canada has 247,000 kilometres of marine coastline; along the southern portions of that coast, numerous industries and municipalities discharge large volumes of liquid wastes into adjacent ocean waters (Figure 1). To properly determine, confirm or predict if these discharges are having an impact in Canada's coastal waters, toxicity tests at source and "in-situ" using marine species must be used. Unfortunately, Canada has fallen far behind a number of countries in this area of expertise over the past decade.

This paper discusses the history and rationale behind using marine toxicity tests for assessing and controlling effluents discharged to marine waters. The use of such tests in other countries and Canada to manage effluents is briefly described. There are several new regulatory initiatives under way in Canada at this time which will require the application of marine aquatic toxicity tests. Efforts are under way in both the public and private sectors to improve Canada's abilities with marine bioassays. The direction and progress of this effort are described.

HISTORY AND RATIONALE OF MARINE TOXICITY TESTING

There has been a major international focus on land-based marine pollution (LBMP) and the necessary strategies and instruments for its control. One recent initiative was the formulation of the UNEP 1985 "Montreal guidelines for the protection of the marine environment against pollution from land-based sources"; they contain a comprehensive environmental protection framework and give clear recognition to the important role of ecotoxicology and biological testing/monitoring in any control strategy addressing LBMP (UNEP 1985, Wells and Côté 1988). Many other agreements and conventions over the past 3 decades, such as MARPOL 73/78 and the London Dumping Convention, also recognize the critical role of biological/toxicity testing and marine hazard assessment in preventing and controlling marine pollution.

Recently, the United Nations is preparing for the 1992 Conference on Environment and Development. GESAMP (United Nations Joint Group of Experts on Scientific Aspects of Marine Pollution) has identified some of the fundamental scientific

FIGURE 1 : DISCHARGES TO THE MARINE ENVIRONMENT IN CANADA

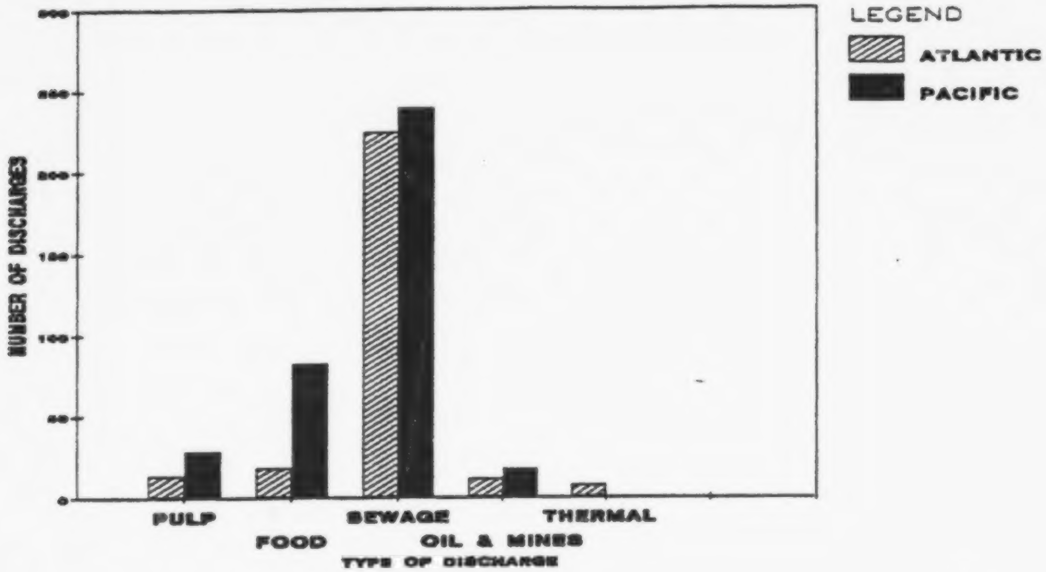
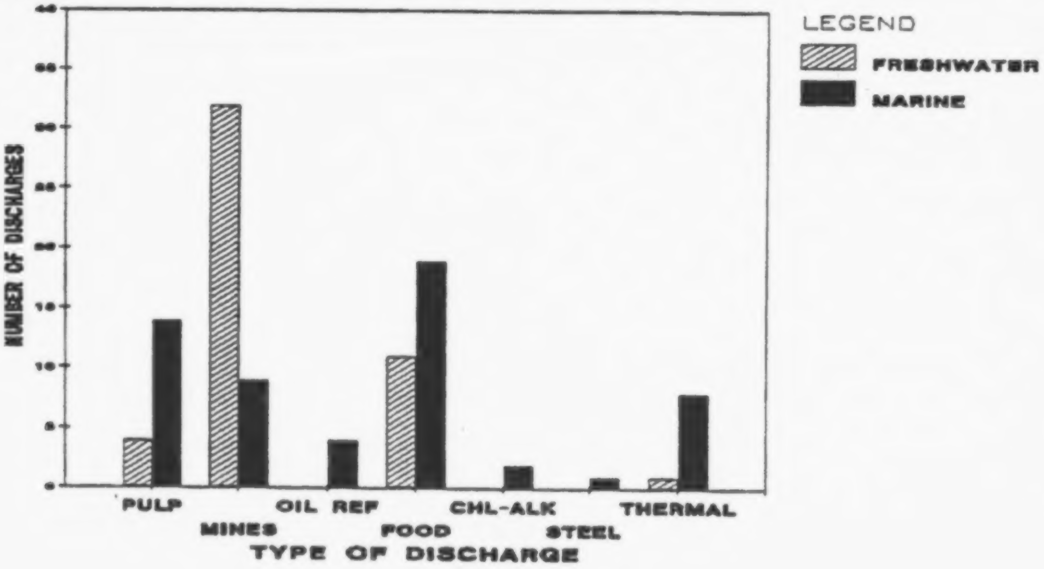


FIGURE 2 - DISCHARGES TO THE MARINE ENVIRONMENT IN ATLANTIC CANADA



principles and concepts to include in any LBMP strategy. The application of current ecotoxicological methods in marine pollution assessment and monitoring is recognized as essential to any chosen strategy (GESAMP 1990).

At the same time, other international organizations and other countries are rapidly recognizing and adopting marine biological tests in their assault on LBMP and other marine pollution problems. For example, the Organization for Economic Co-operation and Development (OECD) sponsored the 1984 International Workshop on Biological Testing of Effluents (and related receiving waters) (OECD 1984, 1987). The OECD Water Management Policy Group and the Expert Group on Biological Testing of Effluents concluded that "toxicity testing can be used successfully for measuring the acute and chronic toxic effects of effluents on the biota of receiving waters, and to generate the quantitative data on effluent toxicity which provide the regulatory basis for the control of toxic substances" (OECD 1987). Canada participated fully in the expert group leading to these conclusions (Blaise et al. 1985, Dafoe et al. 1987, Blaise et al. 1988). In addition, the European Economic Community established an ecotoxicology advisory group in the 1980's, in part to control industrial discharges (Persoone, pers. comm.); there is an aggressive program in some European countries to apply toxicity tests in regulatory schemes for industrial effluents (Hansen, pers. comm.).

The United States now monitors and controls industrial effluents through their National Pollutant Discharge Elimination System (NPDES) program with the States (Federal Register 1984, EPA 1985a), emphasising an integrated approach with whole effluent biological testing and evaluation of the effects of discharges on receiving waters (Degraeve et al. 1988). For example, states such as California and New Jersey have pursued this approach for the control of sewage and industrial wastes. The United States activity has been described in depth at recent SETAC meetings.

Many other countries, such as Australia, Japan and Brazil, are applying biological tests wherever possible in their effluent control strategies. This trend is bound to continue. Hence there is a strong international acceptance of the role of toxicity testing in pollution control strategies, and continued progress on methods and their application.

How have biological tests been applied in Canada? The history, application and continued use of aquatic toxicity tests in Canada, especially for the control of effluents, have been exhaustively summarized over the past decade (Addison 1984; Blaise et al. 1986, 1988; Dafoe et al. 1984, 1987; Day et al. 1989; Degraeve and Wells 1986; MacGregor and Wells 1984; Pessah and Cornwall 1980; Scroggins 1989; Sergy 1987; Van Coillie et al. 1984; Wells 1985, 1989, 1990). Their use within the regulatory framework for effluent control is widely accepted by Canadian biologists and toxicologists, and other practitioners worldwide (see Porcella et al. 1986), even with their known scientific limitations and uncertainties. In Canada, biological tests and the need for aquatic toxicity data have become accepted again by senior environmental managers (Day et al. 1989; Sergy 1987), after more than a decade of shifting regulatory strategies, and inadequate resourcing.

Toxicity tests, primarily acute, have been and are being used in Canada to investigate, regulate and monitor effluents discharged into saltwater. The standard rainbow trout test with Salmo gairdneri (now Oncorhynchus mykiss) has been applied to salt-water dilutions of effluents, primarily with the Steelhead variety of the British Columbia coast. Various estuarine fish, especially the threespine stickleback, have been used to estimate the acute toxicity of effluents. Invertebrates, such as crustaceans (brine shrimp, mysids, decapods) and echinoids, have at times been incorporated into investigations. A salt-water bacterium is used in the Microtox^R test. Other species, as described below, have been used. However, in the regulatory laboratories, marine species work has not proceeded rapidly, even with the recognition in the 1970's that marine tests would be required for definitive data on effects and recovery in the immediate receiving zones of effluents (Pessah and Cornwall 1980).

Canada is currently focusing on the need to control major sources of land-based marine pollution more effectively, especially point-sources such as pulp and paper mill effluents. Federally, Environment Canada is implementing a long-term Marine Environmental Quality Action Plan, involving monitoring, water and sediment quality guidelines, state-of-environment reporting, coastal environmental management programs, research, public information, and enforcement/compliance activities. Nationally there is clear recognition that industrial waste discharges into coastal waters must be more vigorously controlled. There is a growing acceptance of coastal management programs in "areas of concern", similar to the RAP's (Remedial Action Plans) approach in the Great Lakes; such programs are being conducted or initiated in Atlantic Canada, the St. Lawrence estuary as part of the St. Lawrence River Action Plan, and on the Pacific coast. For such programs to succeed, data are required on the acute and chronic toxicities of complex effluents and their key, chemical constituents, at source and under ambient conditions. Such data can only be acquired with appropriate marine toxicity tests with standard and indigenous species. Integrated marine toxicity test approaches, end-of-pipe and receiving water, should be applied to major problem effluents (eg. pulp and paper, McLeay 1987) to ensure accurate predictions of current toxic effects and the positive changes due to effluent treatment.

CURRENT STATUS AND USE IN CANADA

The federal strategy in Canada for regulating industrial effluents uses control at source within major industrial sectors through national Regulations and Guidelines under the Fisheries Act, and more recently under the Canadian Environmental Protection Act. This approach assesses the hazard posed by a discharge using a combination of chemical and toxicity tests. The toxicity tests use "suitable and sensitive" species as surrogates for species found in the receiving environment. The effectiveness of this approach is then intended to be monitored on a site-specific basis (Pessah and Cornwall 1980).

During the 1970s' a series of industrial effluent Regulations and Guidelines were promulgated by Environment Canada under the Fisheries Act. An acute toxicity protocol using fingerling rainbow trout (Oncorhynchus mykiss, formerly Salmo gairdneri), in freshwater was included for the following industrial sectors:

Pulp and Paper
Petroleum Refining
Metal Mining
Potato Processing
Meat and Poultry Products

The objective was to improve final effluent quality so that fish could survive in full-strength effluents, or allowable dilutions, for 4 days (Dafoe et al 1987). The freshwater rainbow trout test continues to be valuable for assessing and regulating effluents in Canada. Present revisions to the Pulp and Paper effluent regulations will require the use of a second test using the freshwater Crustacean Daphnia magna. However there are no marine tests in the industrial effluent regulations and guidelines even though many Canadian industries discharge liquid effluents into coastal or estuarine waters including a high proportion of industries located in British Columbia and Atlantic Canada. For example in Atlantic Canada, all of the oil refineries and 78% of the pulp mills discharge into marine waters (Figure 2). An understanding of the effects of coastal and marine discharges on marine organisms would assist in establishing marine water quality guidelines and would enable regulators to determine the degree of treatment necessary to meet such guidelines.

While marine toxicity tests are not included in effluent regulations and guidelines, they are useful under the General Provisions of the Fisheries Act (Section 36) to determine the deleterious nature of substances (including industrial effluents). These tests are more representative than freshwater tests of conditions found in the coastal and marine receiving environments (Parker 1984 in: Van Coillie et al 1984) and they have been used on a limited basis for many years in Canada to assess and control liquid industrial effluents. In British Columbia, chinook salmon, echinoderm fertilization and Microtox^R tests are used to assess the lethal and sublethal toxicity of effluents discharged into marine waters, with the eventual goal of including some (or all) of these tests in provincial permits (G. van Aggelen, pers. comm.). In Quebec, the Microtox^R assay is used to screen freshwater effluents for toxicity and degradability/persistence of toxicity under the St. Lawrence River Action Plan (N. Bermingham, pers. comm.). In the Atlantic Provinces, Environment Canada uses saltwater fish (threespine stickleback) and Microtox^R assays to assess the toxicity of industrial effluents and also as an aid in prosecutions under the federal Fisheries Act and under various provincial Environmental Protection Acts. In Atlantic Canada, other marine tests have been used occasionally to assess the toxicity of marine effluent discharges eg: gammarid amphipods (required under one provincial permit in New Brunswick); lobster larvae (Sprague and McLeese 1968); marine fish Fundulis heteroclitus and Menidia menidia (EP Atlantic Region, unpublished data).

In the private sector, there have been requests for marine toxicity tests over the past several years (E. Jonczyk of Beak, G. Harris of Harris Industrial Testing, and K. MacPherson of E.V.S., personal communication). These requests have come mainly from the pulp and paper and the metal mining sectors in Canada, as well as from U. S. industries for tests required for N.P.D.E.S. permits. Types of tests requested include stickleback (fish) lethality tests as well as echinoid and mollusc larval assays. Capability to conduct the larval assays does not yet exist in federal government toxicity laboratories, but this is expected to change in the near future.

Canada has fallen behind in its development and use of marine bioassays, and currently has a limited arsenal of standard acute tests. It has no standardized sublethal or chronic marine tests. The regulatory laboratories are catching up now through work on the Core Aquatic Toxicity Tests of the Intergovernmental Aquatic Toxicity Group (IGATG) (see below and other papers at this workshop). However, there is considerable work (adoption of test protocols, QA/QC, etc.)

before the country has a reasonable suite of standardized, single-species, marine tests to apply. Sensitive, multi-species microcosm/mesocosm tests, recommended by many researchers (Cairns 1985, Cairns and Mount 1990), are unfortunately much in the future for routine use.

A summary of the types of marine tests used for effluents in Canada and internationally is provided in Tables 1 & 2.

Table 1: Acute/Lethal Marine Tests Used in Canada and Abroad for Assessing the Toxicity of Effluents

Test Species/Response	Application	Status/References
Threespine Stickleback (*Gasterosteus aculeatus*) (Fish, Lethality)	Effluents, Canadian Fisheries Act	Test procedure in preparation by IGATG[1]
Chinook Salmon (*Oncorhynchus tshawytscha*) (Fish, Lethality)	Effluents	Used by province of British Columbia
Silversides (*Menidia*) (Acute, mortality)	Effluents, Chemicals	EPA 1985
Rainbow Trout >80 g Acclimated to seawater (*Oncorhynchus mykiss*) (lethality)	Effluents, Chemicals	Used in UK (ISO/TC 147/SC 5/ WG 3/N 51)
Lobster larvae (*Homarus americanus*) (Lethality)	Effluents, Chemicals	Sprague and McLeese 1968; APHA[2] et al 1989
Brine Shrimp (*Artemia* spp.) (Crustacean, Lethality)	Effluents, Chemicals	ASTM; Vanhaecke and Persoone 1984; EPA[3] 1985
Mysid (*Mysidopsis bahia*) (Mortality, acute)	Effluents, Chemicals	EPA 1985
Shrimp (*Crangon crangon*) (Acute, lethality)	Effluents, Chemicals	Used in UK (ISO[4]/TC 147/SC 5/ WG 3/N 51)
Mysid Shrimp (*Acanthomysis costata*) Lethality in juveniles	Effluents	California State Marine Bioassay Program
Microtox^{R} (*Photobacterium phosphoreum*) (Bacteria, acute)	Screening of Effluents Chemicals	Test procedure in preparation by IGATG

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1 - IGATG = Intergovernmental Aquatic Toxicity Group (Canadian- Federal/Provincial)
2 - APHA = American Public Health Association
3 - EPA =U.S. Environmental Protection Agency
4 - ISO = International Organization for Standardization

Table 2: Chronic/Sub-lethal Marine Tests Used in Canada and Abroad for Assessing the Toxicity of Effluents

Test Species/Response	Application	Status/References
Sheepshead Minnow (Cyprinodon variegatus) Larval survival and growth	Effluents	Hughes et al 1989; EPA 1988
Inland Silverside (Menidia beryllina) Larval growth/survival	Effluents	EPA 1988
Mysid (Mysidopsis bahia) Survival/growth/fecundity	Effluents	EPA 1988
Echinoderm Various genera (Sublethal-fertilization/development)	Effluents, Chemicals, Fisheries Act EEM Guideline	EPA[1]; ASTM[2]; Dinnel et al 1982; Dinnel et al 1987.
Bivalve embryo/larvae (Mytilus californianus) Sublethal; fertilization and development	Effluents, Chemicals, Sediments	Cherr et al 1990
Red Abalone (Haliotus refescens) Abnormal development	Effluents	California State Marine Bioassay Program
Mussel (Mytilus edulis) Scope for growth Bioaccumulation	Effluents	ASTM; used in the Netherlands
Giant Kelp (Macrocystis pyrifera) Zoospore germination	Effluents	California State Marine Bioassay Program
Red Marine Macroalga (Champia parvula) Chronic effects on reproduction	Effluents	EPA 1988
Kelp (Laminaria saccharina) Effects on reproduction	Effluents	EPA 1989

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1 - EPA = U.S. Environmental Protection Agency
2 - ASTM = American Society for Testing and Materials

IMMEDIATE NEEDS IN CANADA FOR MARINE TOXICITY TESTS

Recent events within Environment Canada's mandate increase the immediate demand for marine toxicity tests.

Revisions are being made to the Pulp and Paper Regulations under the Fisheries Act. Although, the regulations themselves require freshwater acute lethal toxicity tests using rainbow trout and Daphnia magna, a new condition in these regulations will require pulp and paper mills to conduct "environmental monitoring surveys" on a regular basis, probably every three to four years. Since many of the pulp mills in Canada discharge their effluents into estuaries and other coastal waters, it is essential that these "environmental monitoring surveys" include toxicity tests for marine organisms.

Several other initiatives are underway in Canada where marine biotests may be required in the near future for effluent evaluations. On the east coast, two offshore oil production projects have received approval to proceed. The Environmental Impact Assessments conducted for both of these projects require the proponents to conduct environmental effects monitoring on their discharges and in the ocean waters around their production platforms. Although the respective federal-provincial offshore petroleum boards have not completed designs of these monitoring programs to date, biotests will be required (Moores, pers. comm.).

Activities are just under way in Canada to revise the Metal Mining Liquid Effluent Regulation (MMLER) under the Fisheries Act. This regulation came into effect in 1977 and it is anticipated that any revisions will follow the model of the revised pulp and paper regulations. Therefore, it is quite probable that revisions to the MMLER will require environmental effects monitoring to ensure that the regulation is truly providing protection to fish and fish habitat, as broadly defined under the Fisheries Act.

To return to the revisions to the pulp and paper effluent regulations, environmental effects monitoring guidelines (EC,1990) have recently been completed describing how to conduct the surveys. The guidelines establish a core monitoring program which would be mandatory for all mills. The core program is divided into two sections, one for mills which discharge into freshwater and one for those discharging to marine waters. Each core program specifies a series of physical, chemical and biological parameters which must be measured on effluents, receiving waters, sediments or indigenous organisms. Under the present agenda, the guidelines will come into effect in January, 1992.

For marine mills, the "biotests" specified for effluents and receiving water samples are chronic sublethal/lethal toxicity tests utilizing fish, invertebrates and macroalgae (Table 3).

The guidelines also require that genotoxicity tests be conducted on effluent samples. In the receiving waters, mills will also be required to perform in-situ exposures using fish and invertebrates to assess lethality, tainting and bioconcentration of specific organic compounds.

Besides requirements specified for the core program, there are mill-specific monitoring components which may be added onto the core requirements to deal with concerns at specific mills. Among the parameters suggested for the mill-

TABLE 3 : CORE MONITORING COMPONENTS FOR PULP MILLS DISCHARGING TO MARINE WATERS

TEST MEDIA	TEST TYPES	TEST ORGANISMS
EFFLUENTS & RECEIVING WATERS	CHRONIC SUBLETHAL\LETHAL	FISH
		INVERTEBRATES
		MACROALGAE
EFFLUENTS	GENOTOXICITY	BACTERIA
RECEIVING WATERS	IN-SITU EXPOSURES	FISH
		INVERTEBRATES
SEDIMENTS	CHRONIC SURVIVAL/GROWTH	INVERTEBRATES

TABLE 4 : MARINE BIOTESTS FOR PULP AND PAPER EEM CORE PROGRAM

TEST MEDIA	ORGANISM	RESPONSE
EFFLUENTS & RECEIVING WATERS	SHEEPSHEAD MINNOW OR INLAND SILVERSIDE	LARVAL GROWTH AND SURVIVAL
	ECHINODERM	FERTILIZATION
	BLUE MUSSEL	SCOPE FOR GROWTH
	RED ALGAE	REPRODUCTION INHIBITION
EFFLUENT	BACTERIA	AMES, SOS CHROME, MUTATOX
RECEIVING WATER	CAGED FISH	LETHALITY/TAINTING
	CAGED MUSSELS	BIOCONCENTRATION
SEDIMENTS	AMPHIPOD	10 - DAY SURVIVAL
	POLYCHAETE	SURVIVAL/GROWTH

specific modules are acute sublethal toxicity assessments with bacteria and aquatic plants on effluent samples.

Choice of the general types of the above tests took about three years. However, the task of deciding which particular test (species, response, etc.) to put in each slot turned out to be even more complicated; due to deadlines imposed by the regulation revisions process, the task had to be conducted rapidly (Table 4.)

Some of the tests proposed for use in assessing the chronic toxicities of effluents and receiving waters have been adopted from the United States Environmental Protection Agency (USEPA). For fish tests, either the sheepshead minnow or the inland silverside larval survival/growth inhibition test are suggested. These tests are not currently in use in Canada, so Environment Canada laboratories will be required to develop that capability and to assist the private sector to acquire the capability. For marine invertebrate tests on effluent and receiving waters, the biological tests proposed for use are the echinoid fertilization inhibition test and the scope for growth test using blue mussels. The Environment Canada laboratories in the Atlantic and the Pacific and Yukon Regions are currently developing an in-house capability to perform the echinoid tests. For assessing chronic toxic effects on marine algae, a reproduction inhibition test using the red macroalgae, Champia parvula, is proposed. No Canadian laboratory currently performs this test.

To assess genotoxicity of effluents, mills may use the Ames Test, the SOS Chromotest or the Mutatox test. Environment Canada can perform these tests in laboratories in Montreal and Burlington and has extensive experience with them. There is also experience with these tests in the private sector.

For testing receiving waters of pulp and paper mills, two in-situ tests will be required. A 4-day caged fish test will be conducted to assess lethality, tainting and bioconcentration of contaminants. A 21-day exposure using blue mussels will be used to demonstrate bioconcentration and bioaccumulation by invertebrates. Tests of these types have been performed for many years by government and consultants. A standard protocol will be required to ensure a consistent approach to these tests, as for all of the others. Under the site-specific module, mills may conduct a bacterial bioluminescence inhibition test (Microtox^{R}) or a brown kelp reproductive inhibition test using Laminaria spp. The Microtox^{R} test is in common use across Canada in both government and private sector laboratories, whereas the kelp test will require adoption from USEPA.

As shown by this summary, there is considerable laboratory work for both private sector and government laboratories so that all of the above toxicity tests can be performed routinely for all pulp and paper mills discharging to marine waters. Canada has not published protocols for any of these tests. Work on several protocols is currently underway. Most of the proposed tests are currently in use by USEPA and that agency has published protocols for them. Several tests will require a standard protocol to be developed. It is essential that standard, acceptable procedures for all of the tests are available to government and private sector laboratories, as accurate, reproducible and comparable results must be obtained for the 122 Canadian pulp and paper mills. The intent of the revised regulation is to have all mills conducting their own environmental monitoring surveys, probably on contract. Environment Canada laboratories, probably with assistance from provincial toxicity laboratories, will perform audits on each mill's work. To establish the capability of performing all of these tests in both the public and private sectors, a considerable amount of co-operative work must be undertaken. Once an accepted protocol for a particular test has been selected, the technology must be transferred to both government and contract laboratories. Interlaboratory testing will be required to ensure all laboratories are conducting the tests reliably and with known precision. Many of the organisms will be new to the laboratories; reliable sources (natural and cultured) for each organism must be found and laboratory staff must become competent with good culturing, rearing, handling and testing techniques. This will require additional resources for laboratories in both the private and public sectors in order to adopt these new capabilities.

The other key requirement for this initiative is the development of an "assessment document" for all biotests and physical and chemical measurements required by the guidelines. How are the results of each battery of tests to be collectively interpreted? What constitutes an unacceptable effluent or an indication of an environmental impact in the field? For each measured parameter or groups of parameters, pass or fail decision criteria must be established. If an unacceptable environmental impact is occurring, remedial action can then be recommended and pursued. Assessment criteria documents in the USA and Sweden will serve as guides for this effort.

To summarize, the environmental effects monitoring guidelines under the revised pulp and paper regulations will come into effect in January, 1992. That leaves just over one year to select the testing protocols, transfer the technology to both public and private sector laboratories, to conduct the round robins necessary to validate the tests and to prepare the assessment criteria.

SUMMARY AND CONCLUSIONS

The use of acute and chronic marine toxicity tests for identifying, assessing and controlling coastal pollution problems is recognized and practised internationally (Persoone et al. 1984). This is the case for industrial effluents entering estuarine and nearshore waters, especially following the consensus on the approaches reached by OECD in 1984 and 1987.

Hence several decades of scientific and regulatory work have resulted in toxicity being formally acknowledged as a parameter to control at source, and to monitor in the field or with field samples. Toxicity assessments of effluents, dredging spoils, chemicals, formulations and other materials require data based upon exposures of indigenous and standard species, using accepted methods. Hence there is a world-wide effort to choose appropriate testing methods and representative species for acute and chronic tests, especially measuring sublethal effects on critical biological processes. This effort has become quite sophisticated in some countries (eg. Sweden, USA, Japan); advances are being made with both single-species and multi-species approaches, as the ultimate objective is to protect marine ecosystems from long-term impacts of physical and chemical stresses.

Despite long recognition of the importance of aquatic toxicity approaches in the regulatory and monitoring process, including that designed to protect marine ecosystems and marine resources, Canada has fallen behind during the past decade in the selection and application of marine tests for laboratory and field assessments. Although a small number of Canadian research workers have continued to produce, develop and apply field monitoring approaches (eg. Addison and Payne with MFO, Goyette and Vandermeulen with histopathology, Lee with microbial community responses, Chapman with the TRIAD approach, etc), very little progress has been made selecting, standardising and applying a wide range of marine toxicity techniques suitable for laboratory assessment of effluents.

Due to new initiatives (eg. the pulp and paper regulations and effects monitoring guidelines, the need to test and monitor the wastes from offshore oil operations, the need to assess contaminated sediments, etc.), Canada is now having to catch up quickly in both laboratory and field capabilities. Government and private sector laboratories need marine protocols for acceptable tests and approaches, and the capability to perform them reliably. There is an immediate need for more coordinated and co-operative efforts to select and perform such tests, driven by these internal regulatory needs and external expectations of our leadership role in marine environmental protection.

To be successful, additional human and monetary resources will be required for test development and application. This is currently being addressed through the process to revise the pulp and paper regulations, and is considered crucial to the overall advancement of marine approaches.

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EFFLUENT AND AMBIENT TOXICITY PROGRAMS IN THE SAN FRANCISCO BAY REGION

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ABSTRACT

The Regional Water Quality Control Board is conducting an evaluation of toxicity in discharges and receiving waters of the San Francisco Bay region. The ongoing Effluent Toxicity Characterization Program, sponsored by 20 major dischargers, is providing data on species sensitivity, effluent variability and toxicity test precision. The Ambient Toxicity Program has provided data on the spatial and temporal distribution of toxicity at numerous locations near the margins and in the open waters of the estuary. In some cases, effluent toxicity studies were linked to ambient toxicity surveys to assess their value in predicting the potential for receiving water toxicity.

The results of these ongoing programs have demonstrated that toxicity tests are a useful tool in assessing risks of toxicity, even within a complex estuary. Inter-laboratory comparisons indicate that test precision of chronic tests is equivalent to precision of chemical analyses. Furthermore, concurrent effluent and ambient testing have shown that risk assessments based on effluent studies combined with dilution assessments are reasonably predictive of ambient toxicity.

The information gained from both programs is being used to determine the need for Toxicity Reduction Evaluations, to derive effluent limits, and to refine the Region's Toxicity Control Program.

INTRODUCTION

The 1986 Water Quality Control Plan for the San Francisco Basin includes a Toxicity Control Program for evaluating and controlling toxicity associated with point and non-point discharges, including municipal and industrial effluents, dredged sediment disposal, urban runoff, and agricultural drainage. The Basin Plan is developed by the San Francisco Bay Regional Water Quality Control Board (Regional Board), a State agency that regulates water quality of San Francisco Bay and its tributaries within a nine county jurisdiction. The Regional Board receives its legislative authority from two sources: the federal Clean Water Act (through an agreement with the

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Environmental Protection Agency), and from the State of California's Porter-Cologne Act (Water Code).

Two components of the Toxicity Control Program are the Effluent Toxicity Characterization Program, which evaluates toxicity of municipal and industrial effluents, and the Ambient Toxicity Program, which surveys toxicity in San Francisco Bay waters. These programs are designed to provide data to reduce uncertainties in assessing risks to the estuary due to toxicity.

The Effluent Toxicity Characterization Program is providing data on No Observed Effect Concentrations (NOEC's) of permitted discharges, using both acute and chronic (critical life stage) toxicity tests. NOEC's are then compared with Instream Waste Concentrations (IWC's), to determine the level of risk. We consider the risk of toxicity in the receiving water as significant when the NOEC< IWC. Toxicity Reduction Evaluations are required when the number and magnitude of significantly toxic tests reaches specified levels.

The Ambient Toxicity Program is providing data on toxicity in Bay waters. To date, we have surveyed five bay marshes, twelve open water ("background") locations, one dredging event, and a slough. Four of the five surveyed marshes receive secondary treated wastewater from permitted dischargers, as well as non-point inputs. The fifth marsh is a National Wildlife Refuge and receives pollutants only from non-point sources. Two of the marsh surveys were conducted simultaneously with effluent toxicity studies and dilution assessments to determine the extent to which ambient toxicity could be predicted by effluent toxicity.

METHODS

Effluent Toxicity Characterization Program

To date, twenty major (>20 million gallons per day) municipal and industrial dischargers are participating in the Effluent Toxicity Characterization Program. The Program Guidelines (Anderson et al., 1987), adopted by the Regional Board in 1987, fully describe the various phases of the program, which include a quality assurance test round and two data generation phases, the Screening and Variability Phases. The dischargers within the first group of participants are currently in the Variability Phase of the program. A second group of dischargers will commence the program in July, 1991.

A quality assurance/quality control (QA/QC) test round, conducted in August, 1988, was used to determine the ability of commercial and discharger-operated toxicity testing laboratories to conduct the tests required by the program. Laboratories passing the Quality Assurance round were placed on a list of eligible laboratories for participation in the Program. To obtain Regional Board eligibility, laboratories were required to conduct three chronic tests (fathead minnow, Ceriodaphnia, and either the larval mollusc or sea urchin fertilization tests) simultaneously, using blind toxicants supplied by the Regional Board. Laboratories were required to submit test data within twenty-one days. Eligibility was determined by test results falling within known ranges for the selected toxicants and by the quality of documentation.

QA/QC activities during later phases of the program included distribution of a technical questionnaire soliciting comments on the chronic test protocols and site visits to each of the participating toxicity testing laboratories. Responses to the questionnaire were evaluated and discussed at a follow-up meeting. A technical memorandum, setting forth recommendations and additional protocol requirements was then distributed to all program participants. Site visits included review of facilities and raw data sheets for toxicity tests conducted under this program.

The goal of the Screening Phase was to determine the three most sensitive tests for a particular effuent. Each effluent was screened for toxicity using six acute and five chronic tests, conducted simultaneously. At least one fish, one invertebrate and one plant were required for both the acute and chronic batteries. In addition, a mix of fresh, brackish and marine species was required for screening. The NOEC's generated by the Screening Phase testing were used to determine the three most sensitive tests. These three tests comprised the test battery used for repeated testing during the Variability Phase.

The Variability Phase of the Program was designed to evaluate effluent variability, as measured with toxicity tests. Each discharger is conducting up to eighteen tests over the period of a year, using a battery of the three most sensitive tests, determined from Screening Phase data. The scheduling of the tests is based on an evaluation of existing information on effluent quality, including routine and non-routine monitoring of levels of conventional pollutants, previous toxicity test data, and information on the frequency and potential effects of treatment process shut-downs and maintenance operations.

Study plans for both the Screening and Variability Phases must be submitted by the dischargers for approval by the Regional Board before commencing work. A Screening Phase report and two Variability Phase reports (six month progress report and final report) are also required. These reports summarize toxicity test results, water quality data, and test statistics. In addition, the reports must include raw data (laboratory bench sheets) and computer print-outs of statistical test runs. Toxicity test data must also be submitted in a standardized format on a computerized disk.

Data from the QA/QC round were evaluated to determine laboratory preparedness and inter-laboratory test precision. Data from the screening phase were analyzed to determine if there were trends in the distribution and frequency of acute and chronic toxicity, and to assess the usefulness of requiring a broad spectrum of tests in initial screening. The six month progress reports provided data for interim risk assessments. NOEC values were compared with IWC's for each discharge. For this assessment, our Region defines the IWC as the effluent concentration at the edge of the Zone of Initial Dilution. Discharges receiving less than 10:1 initial dilution received no dilution credits, so the IWC was considered equal to 100% effluent. A Toxicity Reduction Evaluation trigger was established as six significantly toxic tests (NOEC < IWC) or three significantly toxic tests, if the NOEC was less than half the IWC in all three tests.

When final Variability Phase Reports are received, Regional Board staff will evaluate data to develop further recommendations for Toxicity Reduction Evaluations, as well as permit limits and program revisions.

Ambient Toxicity Program

Methods used in conducting ambient toxicity surveys are fully described in Anderson et al.(1990b). As a brief summary, ambient toxicity surveys were conducted once or twice in each of five marshes during 1989. Two of the marshes, Hayward Marsh and Mountain View Marsh, were created from secondary treated wastewater, which flows into San Francisco Bay or a tributary to the Bay, after passing through several basins. In contrast, the marshes surveyed near San Jose/Santa Clara and Sunnyvale are natural marshes that receive advanced treated sewage (secondary plus nitrification and filtration). The San Francisco Bay National Wildlife Refuge is a marsh system that includes several sloughs and a creek, which receive some urban runoff.

Surveys of twelve Bay "background" sites, located in deeper, relatively well-mixed areas, were conducted four times between March, 1989 and April, 1990. In addition, one survey was conducted in the vicinity of a steel refinery (USS Posco) discharge and another survey was conducted during a dredging operation.

Surveys of the two natural (San Jose/Santa Clara and Sunnyvale) marshes and USS Posco were conducted at low, slack tide to characterize "worst-case" conditions. These three surveys were linked to studies of effluent toxicity, conducted by the dischargers' contract laboratories. In addition, the sewage treatment plants at San Jose/Santa Clara and Sunnyvale conducted concurrent dye studies to determine dilution within the survey area.

Surveys of the reclaimed marshes were not linked to tidal flow, since tidal dilution is non-existent (Mountain View) or minimal (Hayward).

Survey design varied among locations. The number of sample stations in the marsh, USS Posco and dredging surveys ranged from six to ten. Twelve stations were sampled during each or the four Bay background surveys. Water from each of the sampling stations was tested using three to five species. Sampling methods also varied, depending on field conditions. Subsurface water was sampled by boat, from shore, or from bridges or piers. Precautions were taken to minimize contamination. Salinity adjustments of the sampled waters were necessary in many cases. These were performed with natural seawater brine, commercial salt formulations and mineral waters.

RESULTS

Effluent Toxicity Characterization Program

Ten laboratories participated in the QA/QC round. Data indicated that the marine and freshwater short-term chronic tests are generally reproducible and relatively precise, despite varying animal stocks, dilution waters and laboratory operating conditions (Anderson and Norberg, 1990).

The coefficients of variation (CV's) for the Ceriodaphnia reproduction and survival test and the fathead minnow growth and survival tests were 29% and 31%, respectively, based on results of all ten laboratories. Six laboratories conducted the echinoderm fertilization and four conducted the mollusc (using oysters) development tests. The CV's for these tests were 16.6% and 37%, respectively.

All ten laboratories passed the QA/QC round, and thus were eligible to participate in the program. This indicated that laboratories in the area were generally prepared to do the work required for the Program. However, QA/QC activities during the Variability Phase, including review of raw data sheets and site inspections, revealed poor performance by one laboratory representing several dischargers. Regional Board staff responded by requiring many test repeats. The justifications for test repeats were: 1) missing data for entire effluent tests, 2) missing data for reference toxicant tests, 3) reference toxicant data that repeatedly showed no dose response, 4) inadequate performance in control treatments, and 5) inadequate efforts to obtain spawning animals for urchin fertilization and mollusc embryo tests.

The technical questionnaire soliciting comments on the test protocols received few responses, indicating a general confidence in the toxicity test methods. However, there were some comments that EPA protocol specifications could be interpreted in more than one way, or that protocols were not specific enough on subjects pertaining to the complex salinity adjustments required to conduct these studies in San Francisco Bay. Recommendations and requirements resulting from this questionnaire were distributed to program participants in a September 1, 1989 mailing.

Results of the Screening Phase are fully described in Anderson (1989). Acute toxicity was observed in more than half of the effluents (five municipalities and six industries) tested during the screening phase. Three of the municipal and five of the industrial effluents were acutely toxic to more than one species. For both municipalities and industries, the average number of species affected was two.

However, the acute tests were generally not as sensitive as the chronic tests, as judged by the NOEC values. For industrial dischargers, acute tests were never among the three most sensitive tests. For municipal dischargers, the sanddab acute test was the most sensitive test for four sewage treatment plants with secondary treatment. Each of these plants conducted Toxicity Reduction Evaluations (TRE's), which determined that the sanddab toxicity was due, in large part, to high levels of unionized ammonia. The other acute tests were generally not among the three most sensitive tests conducted during the screening phase.

The order of sensitivity of the species used in acute testing was determined by ranking the percentage of significant ($LC_{50} \leq$ highest effluent concentration tested) responses observed for each species (Table 1). During the Screening Phase, diatom tests were sometimes considered acute tests and sometimes considered chronic tests. The sensitivity ranking for species tested more than ten times was: sanddab = diatoms > mysid shrimp > rainbow trout > water flea .

Sensitivity of acute tests was next evaluated by ranking the number of sites for which a species exhibited the lowest acute effect level (LC_{50}). This method gave the same ranking as the previous method (Table 1). All of the effluents for which sanddab and Neomysis were observed to be the most sensitive species were municipal effluents.

Chronic toxicity was detected in all but three of the effluent tests. Fourteen of the fifteen effluents which exhibited chronic toxicity were toxic to more than one species. For both the municipalities and the industries, the average number of species affected was three.

Species sensitivity in chronic testing was first ranked according to the percentage of significant (NOEC < highest effluent concentration tested) responses for each species (Table 2). Only tests conducted more than five times were included in this analysis. The ranking was: water flea > sea urchin > mussel embryos > fathead minnow = diatoms > silverside minnow. Sensitivity was next ranked according to the percentage of sites for which a species gave the lowest effect level (lowest NOEC) for a given site (Table 2). The ranking was: silversides > sea urchin = mussel > water flea > fathead minnow > diatom. This evaluation indicated that the lowest ranked species in terms of percentage of significant responses (the silverside minnow) was usually the most sensitive when it showed a significant response. Conversely, the species that responded most frequently (water flea) was not among the most sensitive when it showed a significant response.

Results of the Variability Phase, based on six-month progress reports, are fully described in Anderson et al. (1990a). Based on Regional Board review of these reports, toxicity reduction evaluations (TRE's) may be beneficial at several sites, including two oil refineries and one sewage treatment facility

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Table 1. Percentage of significant responses observed for each acute test conducted during the Screening Phase, and the number of sites at which an acute test was the most sensitive (lowest LC_{50}). Values in parentheses are the total number of times tested. The results for tests conducted > 10 times are listed separately from results of tests conducted $\leq$ 10 times.

Species tested > 10 times:	Percent Response	No of Sites Most Sensitive
Citharichthys stigmaeus (sanddab) (16)	38	3
Skeletonema costatum, Selenastrum capricornutum or Thalassiosira pseudonana (17)	38	3
Neomysis mercedes (mysid shrimp) (14)	36	2
Oncorhyncus mykiss (rainbow trout) (16)	25	1
Daphnia magna (water flea) (14)	14	1
Tested 2-10 times:		
Photobacterium phosphoreum (microtox bacterium) (4)	50*	0
Mysidopsis bahia (mysid shrimp) (2)	50	0
Neanthes arenaceodentata (polychaete worm) (6)	17	0
Palaemon macrodactylus (korean prawn) (10)	0	0
Menidia beryllina (silverside minnow) (2)	0	0

* The percentage of microtox responses may actually be lower, because results were not reported by some dischargers who conducted the test optionally.

with advanced treatment. A TRE is ongoing at one oil refinery, and one sewage treatment facility has completed a TRE. At most sites, however, additional information is needed, either to better define initial dilution at the discharge site or to verify observed effects.

Ambient Toxicity Program

The results of the Ambient Toxicity Program surveys are fully described in Anderson et al. (1990a, 1990b). Briefly, toxicity was documented in marshes and Bay "background" stations, located at a distance from point sources of pollutants. Chronic toxicity was observed at three locations within the National Wildlife Refuge, which receives only urban runoff. Toxicity was also observed at many of the twelve Bay "background" stations during all four surveys, using the echinoderm fertilization test. No toxicity was observed using silverside minnow or mollusc development tests.

Table 2. Percentage of significant chronic responses (number of significant responses divided by total number of tests conducted), and percentage of sites for which a species gave the lowest chronic effect level (NOEC). The values in parentheses are total number of times tested.

	% Response	% Most Sensitive
Menidia beryllina (silverside minnow) (16)	44	57
Strongylocentrotus purpuratus (sea urchin) (13)	77	50
Mytilus edulis (mussel) (11)	73	50
Ceriodaphnia dubia (water flea) (14)	79	27
Pimephales promelas (fathead minnow) (14)	50	14
Selenastrum capricornutum, Skeletonema, or Thalassiosira pseudonana (diatom) (13)	50	0

At Hayward and Mountain View marshes, created from undiluted effluent, toxicity was observed at several locations. Toxicity was most pronounced at Hayward marsh, where acute toxicity was observed for two of the three species tested during two separate surveys, with the effects distributed over approximately half of the basins in the marsh closest to the discharge. A preliminary Toxicity Reduction Evaluation was conducted, and it was determined that at least a portion of the toxicity in the marsh was attributable to unionized ammonia.

A survey of the marsh receiving up to twenty-three million gallons per day of advanced treated wastewater from the city of Sunnyvale showed chronic toxicity in the vicinity of the discharge. The results of the ambient toxicity survey confirmed predictions from effluent tests concerning levels of toxicity (no toxicity vs. chronic vs. acute) and the species that would respond. Chronic toxicity was observed in both effluent and ambient samples using Ceriodaphnia, urchin fertilization and diatom tests; no toxicity was observed in effluent or ambient samples using fathead minnow. In contrast, acute toxicity was observed using Ceriodaphnia in a storm drain sump that discharges into the same area during wet weather. In addition, there was no observed toxicity using diatoms in an ambient sample taken from a downstream tributary to the discharge.

A survey of the marsh receiving 120 mgd of advanced treated sewage from the cities of San Jose/Santa Clara revealed no toxicity at any of the ten stations to a distance of five miles from the discharge. The survey near the USS Posco steel refinery discharge indicated that toxicity was associated with both intake and discharge waters. Toxicity could not be specifically attributed to the treated wastes produced by the refinery. Significantly, acute toxicity was detected in intake water from the Contra Costa Canal, a conveyance system that also supplies drinking water to the county.

DISCUSSION

The Regional Board's effluent and ambient toxicity programs are providing data for assessing the risks of discharging toxic pollutants into San Francisco Bay. The Effluent Characterization Program is currently generating No Observed Effect Concentrations (NOEC's) for twenty municipal and industrial discharges. Toxicity Reduction Evaluations (TRE's) are required when a significant risk to the estuary is indicated by NOEC's that are less than instream waste concentrations (IWC's) of the discharge. To date, data have indicated that toxicity reduction evaluations are warranted for one third or more of the twenty discharges tested in the program (Anderson et al., 1990a).

The Ambient Toxicity Program is providing data on toxicity associated with marshes and open water "background" stations (Anderson et al., 1990b). The surveys have been a useful tool for verifying risk assessments based on effluent toxicity testing and dilution assessments. In three cases, ambient surveys conducted in the vicinity of discharges verified predictions based on effluent testing by correctly predicting the level (no response, chronic or acute response) and the species that responded.

The ambient toxicity surveys documented acute toxicity in a marsh created from secondary treated sewage, a storm drain and a conveyance supplying drinking water. Chronic toxicity was documented at other marshes, including a natural marsh within the National Wildlife Refuge, Bay "background" stations, a slough supplying intake water for a steel refinery and a dredging site. No toxicity was observed in a marsh receiving 120 million gallons per day of municipal wastewater (secondary treated with nitrification and filtration).

These results support the view that wetlands used for wastewater treatment must be carefully evaluated and monitored for effects of toxic pollutants. It is still unknown if toxic pollutants in these systems pose more harm than benefit. Also, these observations underscore the importance to our Region of addressing both point and non-point sources of toxic pollutants in the Toxicity Control Program.

An interesting finding was that, with few exceptions, toxicity at Bay "background" stations was only observed using the echinoderm fertilization assay. During the first of four surveys, almost complete inhibition of fertilization was observed. One interpretation of this finding is that moderate toxicity persists throughout the Bay with more extreme toxicity occurring periodically. The widespread nature of the toxicity, based on the response of only one species, is perplexing but not unprecedented. In the Sacramento and San Joaquin Rivers, toxic effects have been documented along river stretches up to 75 miles in length using ambient toxicity testing (Foe and Connor, 1989). Only one (Ceriodaphnia) of several species tested showed a toxic response.

The second possible interpretation of these data is more speculative, but cannot be ruled out in a complex system such as the San Francisco Bay and Delta. There may be positive interferences in the application of this test to Bay waters, such as toxic effects of substances excreted by marine bacteria, physical effects of colloidal material, or subtle changes in ionic content of sample waters. Possibilities of this nature could be addressed experimentally.

The effluent and ambient toxicity programs have not only provided data to evaluate toxicity in discharges and Bay waters, but have also demonstrated that short-term, chronic toxicity tests, including relatively complex marine tests requiring salt additions, can be routinely performed by commercial laboratories. Inter-laboratory test results for chronic tests, obtained during an initial quality assurance round, demonstrated that test precision was comparable to the precision of chemical analyses, despite the fact that laboratories used different stocks of organisms and dilution waters (Anderson and Norberg, 1990).

However, the poor performance of one laboratory during the Variability Phase of the program indicates that QA/QC activities should continue beyond the initial step of determining the general preparedness of laboratories to conduct the tests required by the program. When contracting with commercial laboratories, dischargers should be aware of the additional challenges faced by laboratories conducting large volumes of toxicity tests.

Dischargers should review test results and supporting documentation, including raw data sheets, as soon as possible after the tests are completed.

Data from the Screening Phase of the Effluent Characterization Program have shown the importance of including a broad range of tests during an initial screening to determine the most sensitive test for each effluent. For the most part, the relative sensitivities of the acute and chronic tests could not have been predicted, based on a general characterization of the discharge, such as municipal vs. industrial or secondary vs. advanced secondary treatment with nitrification (Anderson et al., 1989).

Screening Phase data have also shown that discharges may be acutely toxic to one or more species, even though biomonitoring required by NPDES permits does not detect this toxicity. Our Region's required monitoring involves flow-through testing of a choice of two, out of three, fish species (fathead minnow, stickleback, and rainbow trout). These screening data will be used to re-evaluate the effectiveness of the present acute toxicity monitoring requirements in predicting the potential for acute toxicity in receiving waters.

Two general conclusions can be drawn from the Screening Phase data regarding the relative sensitivity of acute vs. chronic tests and sensitivities of the acute tests, when compared with one another. First, the acute tests were never among the three most sensitive tests (from a field of six acute and five chronic tests) for industrial discharges (all of the algal tests were categorized as chronic tests for this analysis). Second, acute tests were never among the three most sensitive tests for municipal dischargers, with the exception of the sanddab acute test, which was the most sensitive test to several secondary treated effluents. Based on these results, the Program may be revised to eliminate all acute tests from the Screening Phase. The sanddab test may, however, be required for some dischargers in compliance monitoring.

Toxicity reduction evaluations conducted in response to the observed acute toxicity to sanddabs indicated that the cause was, primarily, ammonia. However, these evaluations also demonstrated that methods used in conducting this test contributed to levels of unionized ammonia that would not be expected in receiving water. More specifically, the process of mixing hypersaline brine with effluent causing an upward shift of pH. This, in turn, increased the proportion of unionized ammonia in the test containers. One discharger also reported, however, that sanddabs responded to levels of unionized ammonia that were lower than those reported for other sensitive organisms cited in the EPA ammonia criterion document. The sanddab acute test may, therefore, be a sensitive tool for tracking ammonia toxicity problems, if they exist, in estuarine receiving waters.

Since few dischargers have submitted final reports, it is not yet possible to fully analyze Variability Phase data. The data will be used not only to evaluate the variability of effluents, but also to assess variability in test precision. Of particular interest is how precision of the echinoderm fertilization and mollusc larval abnormality test may be affected by reproductive seasonality. These analyses will be important in establishing reasonable specifications (e.g., monitoring frequency, species, dilutions, etc.) for permit limitations.

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GROWTH INHIBITION TEST WITH FRESHWATER ALGAE: ENVIRONMENT CANADA'S REFERENCE METHOD

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With ever-increasing demands being placed on environmental laboratories for cost-effective biological tests, the microplate growth inhibition test using Selenastrum capricornutum (Printz) has been selected as one of the national core aquatic toxicity tests. Standardization of the bioanalytical protocol then became imperative so as to maximize its usefullness, notably as a potential regulatory tool. The different aspects of the algal microtest, resulting from this standardization exercice, are precisely defined.

Thus, a more rigorous washing method is created for the removal of labware-attached trace metals and organics which are likely to affect algal growth. The specific nature and concentration of micro- and macro-nutrients are determined. The latter spike the test treatments and controls to ensure that absence or inhibition of growth after biotesting can be unambiguously linked to a toxic effect and not to a nutrient limitation of the sample. The presence of EDTA (ethylenediaminetetraacetate) in the microtest medium is recommended to increase bioassay reproducibility. EDTA, acting as a chelator, keeps trace elements available and captures the organic metabolites excreted by algae which may otherwise become of decisive importance regarding the final toxicity. Similarly, sterilization of the medium by filtration exclusively is also recommended.

A standard microplate configuration, which corresponds to a particular assignment of the wells in the microplate, is conceived to best reduce the variability among replicates. This standard configuration allows, moreover, to control for the presence of volatile toxicants contained in a sample. Indeed, with such a configuration, a significantly reduced or increased growth and/or heterogeneity in control cell counts indicates that a contamination attributable to vapors emitted from the sample very likely occurred during the incubation period. For testing such volatile substances, a specific procedure is described.

In addition to industrial and sewage effluents, surface and groundwater, contaminated sediments and landfill leachates, chemicals and products, pesticides, airborne contaminants, developmental chemicals or formulations, and spilled substances or those likely to be spilled, the applicability of the microtechnique to substances with limited water solubility is confirmed. These hydrophobic substances may require an incorporation into organic solvents in order to solubilize. Consequently, another specific procedure within the reference method, incorporating additional controls to account for the presence of the solvent, is presented.

The selected endpoint for the algal toxicity microtest is the IC_{50}, defined as the sample concentration causing a 50% reduction in the growth of the algal population. Furthermore, the standard response variable from which the endpoint is estimated becomes the growth rate. The growth rate, simply defined as the change in biomass of the algal population divided by the duration of the test, is a measurement of a global rate of change. It is different from the specific or intrinsic growth rate which measures the rate of change at an instant in time. Because the growth rate is obtained directly from mathematically untransformed count-generated cell densities, it is simpler to calculate and more sensitive than other parameters. Due to its high sensitivity, the growth rate is preferred since it generates a more cautious estimation of the potential hazard of the water sample.

Using the selected response variable, the most adequate method of calculating the IC_{50} is described. It consists of plotting the arcsine transformed "percentage growth inhibition" vs "sample concentration" curve. The endpoint is then estimated by inverse prediction, i.e. by intrapolating from the simple regression line fitting the curve. To further increase the precision of the IC_{50} value, a toxicity assessment scheme involving preliminary range-finding tests and a definitive test is also delineated.

In addition to standardizing other methodological steps (e.g. test organism revitalization process, test conditions, choice of test concentrations, selection of dilution water, etc.), quality control practices for substantiating the validity of analytical data are also specified. For example, copper sulfate, potassium dichromate, zinc chloride and phenol are the reference toxicants designated to demonstrate, using the X-control chart technique, the ability of laboratory personnel to obtain consistent, precise results.

Finally, the specifications of the algal bioassay protocol have been defined so that the results obtained may be still more accurate and reliable. They result from the current knowledge of scientists and protocol development officers and may become inadequate as new discoveries are made. Should that arise, improvements can always be made and the present standardized methodology will have served, at the very least, as a starting point.

IDENTIFICATION AND CONTROL OF BIOCONCENTRATABLE CHEMICALS IN EFFLUENTS.

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A guidance document has been prepared to assist in developing NPDES permits for the control of bioconcentratable (and bioaccumulatable) pollutants in effluents. This document contains procedural recommendations for identifying and limiting individual bioconcentratable pollutants in complex industrial and municipal effluents. The guidance provides 1) a rationale for the control of chemicals that bioconcentrate which do not exhibit acute or chronic toxicity, 2) an analytical procedure for identifying bioconcentratable chemicals in effluents, and 3) a method for establishing reference ambient concentrations which limit human exposures to unsafe contaminant levels in fish and shellfish.

The guidance, the assumptions and underlying principles employed by the guidance, problems in development of the guidance, limitations of the guidance, and some field validation results for the guidance will be presented and discussed.

TOXICITY IDENTIFICATION/REDUCTION EVALUATIONS: A DEVELOPING SCIENCE.

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SUMMARY

The U.S. Environmental Protection Agency (EPA) published in 1984 a national policy for development of water quality-based permit limitations for toxic pollutants (Federal Register, 1984). EPA subsequently published a technical support document to provide procedural recommendations for controlling adverse water-quality impacts on receiving waterbodies caused by discharge of toxic pollutants (EPA, 1985). When effluent dischargers, permitted under the National Pollutant Discharge Elimination System (NPDES), cannot achieve effluent toxicity limitations, EPA or state regulatory agencies may require the discharger to conduct a Toxicity Identification/Reduction Evaluation (TI/RE). A TI/RE is a step-wise evaluation intended to determine those actions required to achieve compliance with water-quality based effluent limits. Figure 1 (adapted from Fava et. al., 1989 and Botts et al., 1989) provides a conceptual overview of the TI/RE process indicating the steps required to characterize, isolate, and identify the source(s) of effluent toxicity. Major TI/RE steps are: 1) acquisition/analysis of site-specific information, 2) evaluation of housekeeping, treatment plant operation, and chemical use, 3) toxicity identification evaluation (TIE), 4) source identification, 5) toxicity reduction methods evaluation, and 6) confirmation of toxicity reduction. The first task of a TI/RE is to utilize all of the available information in developing a candidate list of suspect toxicants. This is an important ongoing task at the facility to assess potential sources of toxicity, and evaluate the general housekeeping practices, chemical usage, and operation of the wastewater treatment plant. A second step in the initial evaluation is to evaluate effluent toxicity variability and determine if there are consistent differences in species sensitivity to the toxic agent(s).

The objective of the TIE component is to identify, or at a minimum characterize, the toxic agent(s) in the effluent, so that the source(s) of the toxic agent(s) can be identified and an appropriate strategy implemented to reduce effluent toxicity to an acceptable level. This is an evaluation, which, depending upon the results, could be completed after any one of the following sequenced steps:

- Characterization of effluent toxicity by general chemical class.
- Evaluation of the role of candidate toxicants which are identified in the analysis of the existing data and detected by class in toxicity characterization/fractionation efforts.
- Identification (to extent possible) of the toxicant or toxicants.
- Confirmation of the identified toxicant(s).

TI/RE FLOWCHART

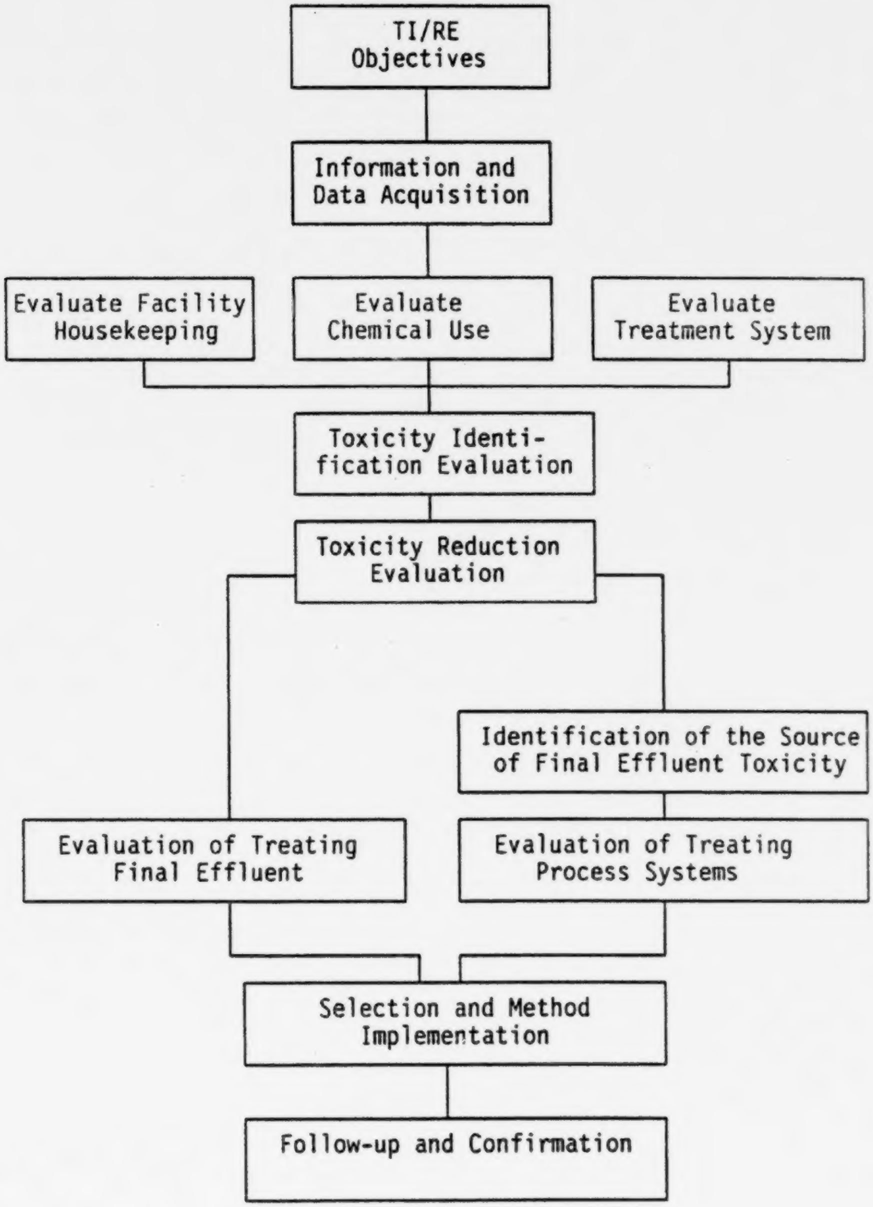
TI/RE Objectives
Information and Data Acquisition
Evaluate Facility Housekeeping
Evaluate Chemical Use
Evaluate Treatment System
Toxicity Identification Evaluation
Toxicity Reduction Evaluation
Identification of the Source of Final Effluent Toxicity
Evaluation of Treating Final Effluent
Evaluation of Treating Process Systems
Selection and Method Implementation
Follow-up and Confirmation

Figure 1. GENERALIZED TI/RE FLOWCHART

The effluent toxicity is first characterized to determine the general chemical class of the toxicant or toxicants. Characterizations can be performed using procedures developed by EPA (Mount and Anderson-Carnahan, 1988) and others to determine if the effluent toxicity is associated with:

- filterable materials,
- organic or inorganic (anionic or cationic) compounds,
- volatile organic compounds,
- compounds with toxicity affected by pH,
- oxidizable or reducible compounds,
- persistent or non-persistent constituents, or
- chelatable metals.

The EPA methods are used to characterize the physical/chemical nature of the causative toxicants in an effluent and to assess the variability in the characteristics of the toxic constituents. A number of characterizations, over time, may be needed to characterize the nature of the effluent toxicity. Following isolation of the toxic fraction or fractions, the specific toxicant or toxicants are identified using EPA Phase II - Toxicity Identification Procedures (Mount and Anderson-Carnahan, 1989). Chemical identification can usually be performed using accepted standard analytical chemistry techniques, although novel analytical techniques may need to be employed for some toxicant identifications.

The final step in the TIE is to confirm the toxicants which have been identified. The methods used for the confirmation of the constituent(s) responsible for toxicity are those included in EPA's Generalized Methodology for Conducting Industrial TREs and those presented in EPA's Phase III document - Toxicity Confirmation Procedures (Mount, 1989). No single confirmation method produces conclusive evidence; therefore, multiple confirmations should be employed. Toxicant confirmation is a critical and often overlooked step in the TI/RE process. The role of any candidate toxic agents identified in the data review and detected in the toxicity characterization efforts can be confirmed by the following procedures:

- Review existing data in the aquatic toxicology literature to ascertain if the suspect toxic agent(s) are likely to be toxic to the test species at the levels found in the effluent.

- Remove the suspect toxic agent(s) from the effluent and determine if some or all of the toxicity is removed by this step. Conversely, restore the toxic agent(s) to the effluent and ascertain whether the toxicity returns at the anticipated levels.

- vary the levels of the suspect toxic agent(s) in the effluent to determine if there is a correlation between the concentration of the toxicant and the effluent toxicity.

- evaluate responses of different species known to have varying sensitivities and toxicity symptoms to the suspect toxic agent(s).

No single procedure is adequate to provide confirmation of the suspect toxic agents; several procedures should be used to obtain sufficient "weight of evidence" for the final stage of the TIE.

The TRE phase can only be initiated following completion of the toxicity identification phase. The scope of the toxicity reduction phase is highly dependent upon the results of the TIE and typical TIE scenarios are:

- A readily identifiable toxic agent or a limited number of toxic agents are identified.
- A single class of substances (i.e., volatile organics, chelatable metals or reducible substances) are identified as consistently causing toxicity.
- No single class of substances are consistently correlated with effluent toxicity.

As a result of the TIE phase, it may be possible to readily identify a single compound or a limited number of substances as the cause of toxicity. If the toxic agent is a rather ubiquitous substance(s), such as a single metal, then the sources of the toxic agent can be identified by analyzing for the toxic agent in the major influent streams flowing to the treatment plant from the wastewater streams at the facility. These results can be evaluated, and based on a mass balance determination, process stream concentration limits can be applied for those specific constituents. The characteristics of each wastewater stream may not allow pretreatment at the source; however, product substitution or process modification may be alternative toxicity reduction options.

Because causes of aquatic toxicity are often complex, it is frequently difficult to identify specific individual toxic agents. However, effluent toxicity may be limited to a particular class or classes of substances, such as chelatable metals, nonpolar organic compounds or volatile organic compounds. A specific class of substances may consistently be identified in the toxicity characterization/fractionation procedures, and depending upon the particular toxic fraction or fractions, it may be possible to identify the source(s) of the toxic agent(s). For instance, if the toxic fraction is a nonpolar organic compound, and GC/MS identification suggests that the toxic fraction is a mixture of compounds commonly used at the facility, chemical use data from the various processes could be reviewed; if the compounds are present in the effluent, then these materials could be traced to their source(s) and their contribution to toxicity could be confirmed. In addition, individual percentages of toxicity contributed by each substance to the overall effluent toxicity could be determined.

If the TIE determines that effluent toxicity is caused by a wide variety of substances, or is highly variable (both quantitatively and qualitatively) over time, source identification and subsequent reduction can be highly complex. In these cases, it may be necessary to utilize toxicity testing alone to track the toxicity to its source. This approach may be difficult because toxicity in an untreated process discharge to the wastewater treatment system may not necessarily translate to toxicity after treatment.

Many toxicants are highly treatable and may be routinely removed by the facility's treatment process. Thus, even if an influent stream to the wastewater treatment system is toxic, the influent stream's toxicants may be readily removed by the wastewater treatment plant, and may not be responsible for effluent toxicity. Consequently, it may be necessary to confirm individual streams as contributors to effluent toxicity by either testing suspect process streams to verify their contribution of toxicants to the wastewater treatment plant, and/or by identification of the toxic process stream(s) by selective temporary isolation of each wastewater stream within the collection system. Toxicity tracking may also be accomplished using pilot-scale treatment for determining the source(s) of pass-through toxicants. Other alternatives for toxicity reduction are provided by EPA guidance documents (EPA, 1989) including options such as process modification, chemical substitution, and improved housekeeping practices.

In general, removal of toxicity at its source is usually preferable to end-of-pipe treatment. This approach is consistent with EPA policies on waste minimization and pollution prevention, and may be a more economical and effective alternative to end-of-pipe treatment. TI/RE procedures provide a useful framework for characterizing, isolating, and identifying toxic agents in effluents and these procedures can be use to implement waste minimization and pollution prevention programs at industrial and municipal facilities.

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GUIDANCE ON CONTROL OF TOXICITY TEST PRECISION USING REFERENCE TOXICANTS

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Abstract

Toxicity test data generated in a regulatory environment that do not agree among laboratories will complicate interpretation of compliance versus non-compliance. Quality Assurance/Quality Control (QA/QC) programs will be necessary to ensure that comparable results can be achieved by different laboratories. One component of toxicity laboratory QA/QC is the control of intralaboratory test reproducibility (precision) using reference toxicants.

For each type of test conducted in a laboratory, a reference toxicant should be selected and tested on a routine basis (eg., at least once per month). Temporal variability in test results can be monitored using mean control charts. High versus low plots and range-type control charts can be used to monitor both spacial and temporal variability of duplicate effluent or reference toxicant test results. Test results which fall outside warning or control limits on a control chart indicate a potential problem in the test system, and warrent immediate investigation. If the outlier is attributed to a factor (eg., poor organism health) which may affect other, concurrent tests of that type, those tests must be repeated or the results reported as suspect until the problem is corrected and an acceptable reference toxicant test result is obtained.

INTRODUCTION

Toxicity tests are becoming increasingly important in the assessment and control of substances discharged by industrial and municipal facilities to Canadian surface waters. Test data generated in a regulatory environment that do not agree among laboratories will complicate issues of compliance versus non-compliance. Implementation of rigorous quality assurance/quality control (QA/QC) programs in toxicity laboratories will be necessary to ensure that comparable results can be achieved by different laboratories. Two aspects of toxicity laboratory QA/QC can be addressed by the use of reference toxicants.

A reference toxicant is simply a chemical that is used in toxicity testing in order to make comparisons between test results. One function of reference toxicants is to judge comparability between laboratories. The second function of reference toxicants is to provide a general measure of the reproducibility (precision) of a toxicity test method within a single laboratory over time. Since the two functions of reference toxicants address two separate components of laboratory QA, the necessary characteristics of the substances used for each function differ. Some, but not all, reference toxicants will share the characteristics required for both purposes. This study addressed only within-laboratory control of toxicity test precision.

Other elements of precision control in the laboratory do not involve reference toxicants (eg., duplicate testing of actual samples such as effluents). These methods are discussed and contrasted with reference toxicant methods.

ESTABLISHING AND UPDATING CONTROL CHARTS

Reference toxicant tests are used to demonstrate the ability of laboratory personnel to obtain consistent, precise results with a given test organism and protocol. This can be accomplished using the same techniques that have been developed by chemical analytical laboratories over the last 20 years. One of these techniques is the mean chart (Dux 1986).

The mean chart is prepared for reference toxicant tests by plotting results of successive tests on a chart, where the x-axis represents test date or test number and the y-axis indicates end-point concentration. Point estimates, such as LC50 or ICp, are appropriate for charting, in contrast to discrete variable end-points, such as NOEC or Chronic Value.

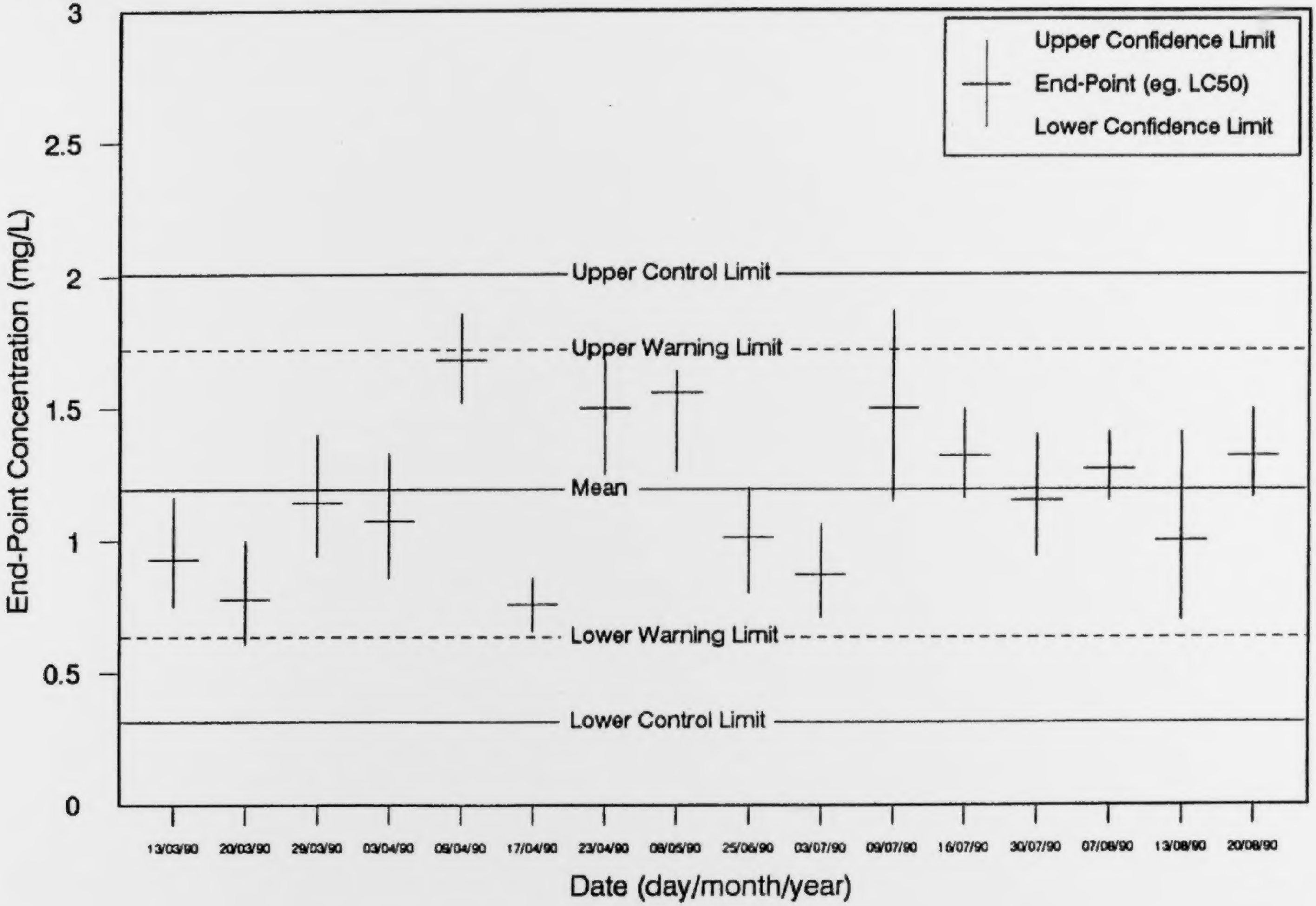
The mean and standard deviation of a set of reference toxicant test data can be used to define a range of "normal" or "acceptable" variability in the test. The concentrations which equal two times the standard deviation above and below the mean represent the upper and lower 95% confidence limits, respectively, for that data set. These lines are then plotted on the mean chart as Warning Limits (Figure 1). The concentrations which equal the mean plus or minus three times standard deviation represent the 99.7% confidence limits ("Control Limits").

The data can be stored electronically using commercially available spreadsheet software such as Lotus-123 or Multiplan to facilitate re-calculation of the mean and standard deviation for each data set. The charts can be plotted manually, but can be more conveniently plotted and updated using software packages such as Microsoft Chart, Lotus Freelance, or Harvard Graphics.

REFERENCE TOXICANT SELECTION

The criteria considered to be important in identifying suitable reference toxicants have varied between authors (Alderdice 1963, Adelman and Smith 1976, LaRoche *et al.* 1970, Fogels and Sprague 1977), depending largely on each author's perception of the underlying objective(s) of the tests. The following eight criteria were identified by the authors of this study as desirable properties of any chemical to be used as a reference toxicant in monitoring toxicity test precision within a laboratory.

FIGURE 1: MEAN CHART



- Tests can detect abnormal test organisms;
- Intralaboratory water quality variations have a limited effect on variability of test results;
- Established toxicity database;
- Readily available in pure form;
- Easily analyzed;
- Soluble;
- Stable in solution;
- Stable shelf life.

Although the rationale for each criterion is self-evident, two points merit additional discussion.

The ability of reference toxicant tests to detect abnormal organisms has often been cited as an important, if not primary, purpose for the tests. Literature searches and personal contacts revealed that alarmingly little research has been conducted to verify that any reference toxicants are able to consistently reflect poor organism health or genetically different stocks.

Also, two alternative approaches are possible with respect to the effect of variations in laboratory water quality on toxicity test results:

- the toxicity of the reference toxicant should be highly sensitive to water quality in order to monitor the extent to which other test results may be affected; or
- the toxicity of the reference toxicant should not be sensitive to normal water quality changes within a single laboratory in order to isolate the effects of other sources of variability (eg., organism health, technician performance).

The first approach is not practical, since no single toxicant will give toxicity test results that reflect all water quality changes, nor could the degree of response to water quality changes be extrapolated to other types of test solution (eg., effluents).

Once a chemical has been selected based on the above criteria, it must be evaluated with respect to specific test organisms and protocols. In particular, the toxicant mode of action is important; a chemical that does not reach a toxic threshold within the test duration will likely show greater variability in test results over time than is reflective of typical laboratory performance. In addition, for reasons which are not yet clear, some chemicals appear to work better in some laboratories than others; possible factors include laboratory water quality and genetic tolerance of organisms.

The results of an evaluation of eight reference toxicants for use in seven test types has been presented in an Environment Canada document (Environment Canada 1990).

TESTING FREQUENCY

Ideally, reference toxicant tests would be conducted continuously for each test type that is being performed by the laboratory to minimize the time lag involved prior to detection of an abnormal condition. However, this frequency is impractical for most laboratory operations. The appropriate testing interval should be determined by experience gained in developing a base of reference toxicant data (e.g., after 15 to 20 tests). It is recommended that monthly testing be conducted as a minimum. For organisms that are not cultured in the laboratory, an additional stipulation is that all stocks be tested upon arrival and just prior to exhaustion of the stock.

Reference toxicant tests should be conducted more frequently when new organisms or protocols are introduced into the laboratory in order more quickly to establish representative control limits. Weekly tests are probably not unreasonable for acute tests and bi-weekly for chronic tests in the initial few months of testing. All laboratories would be well advised not to report the findings of new tests until consistent reference toxicant test results can be demonstrated.

CHEMICAL CONFIRMATION OF TEST SOLUTIONS

Periodic confirmation of test solutions upon makeup is necessary to ensure that accurate toxicant concentrations can be achieved. Samples of exposure solutions should be collected from every test to be analyzed chemically if toxicity results are out-of-control. In addition to out-of-control situations, samples should be analyzed from a low, medium, and high concentration in each type of test at least 2 times per year (approximately once every 6 tests) to confirm that actual concentrations are acceptable representations of nominal concentrations.

The degree of difference that may be considered acceptable will depend on the analytical precision for that chemical at different concentrations. It is advisable to consult with the analytical laboratory to determine the level of difference between nominal and measured concentrations that can be considered significant. Accuracy in solution preparation of 10% should usually be achievable.

Unacceptable deviations in measured concentrations from expected concentrations will require a thorough investigation to identify the source of error. Calculation errors, dilution errors, poor accuracy in instrumentation and equipment are potential factors that will result in inaccurate solution concentrations.

STATISTICAL CONSIDERATIONS

One of the assumptions underlying control charting statistics is that a sufficient number of tests has been properly conducted to give a representative range of variability. To be certain that this is the case, a minimum of 15 to 20 tests may be necessary (Dux 1986). This may require a considerable period of time (particularly for chronic tests), and the toxicity laboratory will probably wish to estimate their test precision prior to that time. The U.S. EPA requires that a minimum of 5 tests be conducted before 95% limits are established (Weber et al 1989). The laboratory should be aware that until a large number of tests have been completed, the limits are likely to change with the addition of each new data point to the data set. Over time, the limits will then stabilize.

Another statistical assumption is that the data are normally distributed. The Kolmogorov-Smirnov test for normality (Sokal & Rohlf 1981) should be performed prior to establishing a control chart. If the raw data are normally distributed, the arithmetic mean and standard deviation are used. Otherwise, a transformation must be performed to normalize the data. In the authors' experience, log transformation will usually result in normality of non-normal LC50 data. Such data maybe charted on the transformed or arithmetic scale; if log data are plotted on the arithmetic scale the associated 95% and 99% confidence limits will not be equi-distant about the mean.

If a logarithmic transformation does not result in data normality, a suitable transformation must be found. The laboratory may wish to follow the maximum likelihood method of Box & Cox (1964) for choosing an optimum transformation.

Another consideration in the establishment of control charts is that data which show a high degree of variability will result in a large standard deviation about the mean, causing control limits to be wide. Therefore, although a laboratory may not be generating consistent results, they may be able to demonstrate that the data are within the Warning Limits. There do not yet appear to be any accepted standards regarding the width of the 95% confidence limits among regulatory authorities that have implemented reference toxicant testing requirements (e.g., U.S. EPA). The authors suggest that each laboratory set an objective coefficient of variation (% CV = 100 SD /mean) of 20% for each test. It is recognized, however, that such factors as the degree of standardization of each test protocol will also affect test reproducibility. A higher CV (e.g., 30%) may be more realistic for some tests. It will not be possible to set specific limits on the width of control limits until sufficient data have been collected and reviewed to determine the degree of reproducibility that can be achieved.

DATA INTERPRETATION

Warning Limits

At the 95% confidence level, 5% of the test results would be expected to fall outside of the Warning Limits due to chance. An outlier should prompt a review of the test system. A mistake in stock solution preparation, a dilution calculation error, stressed or undernourished test organisms are only some of the possible factors. It is particularly important to examine other measures of QA/QC in the laboratory. Control survival during the tests, reproductive success of cultured organisms, time-to-first-brood and size of first brood in invertebrate cultures, dissolved oxygen levels, test temperature, etc., will provide important clues as to whether the outlier occurred by chance or, more likely, was due to a change or problem in the test system.

Separate control charts should be prepared for each reference toxicant-test species-test protocol combination. Each new test result (LC50, EC50, or IC50) should be compared against established Warning Limits and, if it falls within the limits, it should be included in the data set. If an outlier (of Warning Limits) is attributed to a specific problem in the test system (e.g., dilution error, miscalculation of data, poor organism health) the data point should not be included in re-calculation of the limits. If the outlier appears to represent normal variability, it should be included in the data set.

The data from other tests of that type which were conducted during the period of time for which the test corresponds may need to be flagged as suspect if the reason for the outlier is not identified, or if it is traced to a factor that was common to the other tests. For example, if an outlying reference toxicant result for *Daphnia magna* was attributable to poor culture conditions at that time (e.g., crowded), then other *Daphnia magna* tests may be suspect. Alternatively, if the outlier was traced to a mistake in the preparation of the stock solution, the results of concurrent effluent tests may be quite acceptable, particularly since test solutions for the two types of tests (effluent vs. pure chemical) are prepared by different procedures. In either case, the test data in question (e.g., effluent test results) should be reported with a note detailing the reference toxicant test results, interpretation, and other relevant QA/QC data.

The reference toxicant datum that is to be reported with each set of routine test data should be that generated by the most recent reference toxicant test. The period of time for which each reference toxicant datum applies is therefore dependent on the chosen testing frequency.

Over time, the frequency of data falling outside the 95% limits should be close to 5%. If the frequency exceeds 5%, miscalculation of the limits or a deterioration in precision is likely indicated. A frequency of less than 5% may also indicate miscalculation of the Warning Limits or may demonstrate improved test precision. In either case, the laboratory may wish to re-establish the Warning Limits based on more recent data in order to more closely monitor and maintain the enhanced precision.

Control Limits

The probability of a datum falling outside the 99% confidence limits due to chance alone is only 0.3% (one out of every 333 tests). Therefore, even if a specific cause cannot be found to account for the outlier, it should not be attributed to chance. The test system should be reviewed and concurrent data for that test system should always be flagged as suspect. The outlier should not be used in re-calculation of 95% and 99% confidence limits.

Data Trends

It is not only important to monitor whether or not each data point falls inside or outside established Warning Limits, but also to monitor trends or patterns that develop in the data. Out-of-control data may be prevented by early detection of a trend.

Probability theory dictates that the probability of any single data point falling above or below the mean line is 50% or 1/2 (assuming random sources of variation). The probability of two consecutive points being on the same side of the line is 25% or 1/4. The probability of n points being on the same side of the line is therefore $1 / (2^n)$. If $n = 5$, the probability is only about 3% that this occurred through chance alone. Therefore, if 5 or more consecutive points are on the same side of the mean line, some action should be taken to detect a source of bias (Dux 1986).

TRAINING NEW TECHNICIANS

Reference toxicant tests can be used to judge the progress of new personnel. New technicians should be required to conduct a series of reference toxicant tests until they are able to demonstrate the ability to consistently generate results within established Warning Limits.

To ensure that each technician in the laboratory is capable of preparing accurate solutions, stock solutions should be prepared by each person at least once annually and submitted for analysis. New personnel should be required to submit a toxicant solution for chemical confirmation early in their training period. The acceptability of each result will depend, in part, on the uncertainty associated with the analytical method.

DUPLICATE TESTING

Mean-type control charts provide a measure of technical and temporal precision within a laboratory. Duplicate testing provides a measure of technical and spatial variability. Since the laboratory will usually be conducting reference toxicant tests once every two weeks to a month , the duplicate reference toxicant testing is probably only practical at a frequency of 3 to 4 times per year. However, duplicate tests should be performed in approximately one out of every ten routine tests in the laboratory. It is appropriate to perform duplicate tests on other types of samples analyzed by the laboratory and to document the results separately from the duplicate reference toxicant test data. For example, since the majority of tests conducted by many toxicity laboratories are effluent samples, it is appropriate to do duplicate effluent testing on those samples.

Two alternative ways by which duplicate effluent test results can be analyzed, and one method for reference toxicants, are discussed below. The laboratory may choose whichever method is most appropriate. A limitation to both effluent methods is that the data points (unless individually identified as a letter, special symbol, or date, for example) do not indicate temporal changes in precision. It is advisable to review the data after every update to identify temporal trends that may indicate the data are moving toward an out-of-control condition.

High vs. Low Duplicate Plots

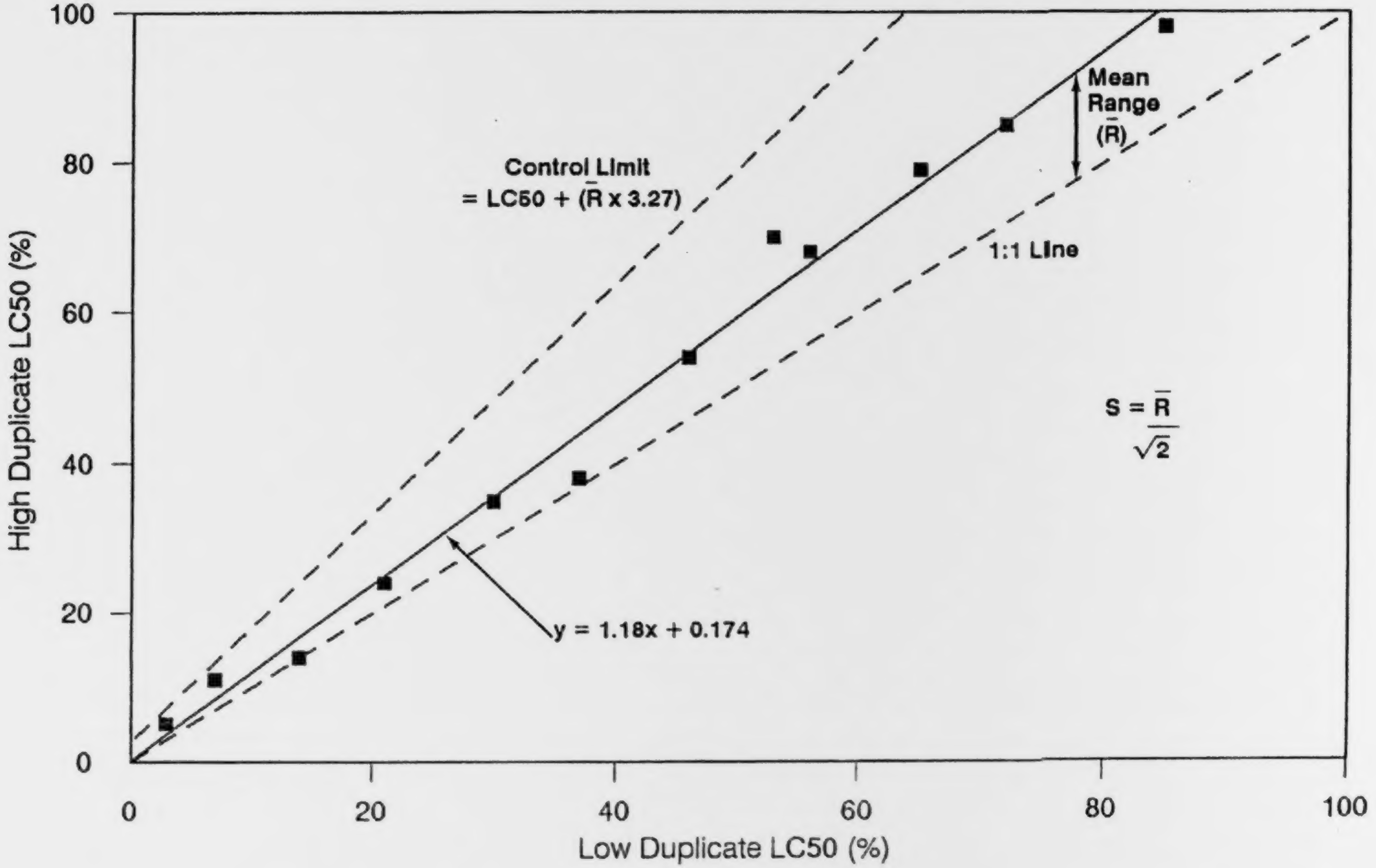
This method is used for effluent test control, when considerable variation in the toxicity of different samples may occur. Duplicate test results (e.g., LC50, EC50 or IC50) are plotted with the lower value on the x-axis and the higher value on the y-axis (Figure 2). The standard deviation associated with duplicate samples at any given concentration is represented by the mean range at that concentration divided by the root of 2. Consequently, if the slope of the regression line fit to this plot is 1, then the y-intercept represents within-laboratory test variation at all LC50 concentrations, ie.:

$$S = \frac{\text{intercept}}{\sqrt{2}}$$

If the slope is significantly different from 1, then there is a systematic change in reproducibility with changing toxicity. In either case, a control limit (99% confidence limit) can be calculated for the data (LC50 (from 1:1 line) + (R x 3.267)), where R equals the expected range (high minus low LC50 at a given LC50 value), and 3.267 is adopted from standard control charting practices (King 1984; ASTM 1986). Substitution of 2.456 in place of 3.267 defines the 95% (warning) limit.

Tests outside the control limit would be suspect. As with mean charts, the statistics are based on the assumption that the data set contains a sufficient number of representative

FIGURE 2: HIGH vs. LOW DUPLICATE CONTROL CHART FOR EFFLUENTS



samples (e.g., 15 to 20), and that residuals from the regression line follow a normal distribution.

Range-Type Control Charts for Effluents

To set up a range-type control chart, the range for each pair of end-points (e.g., LC50) should be plotted against the mean end-point value. The range of difference between duplicates may be dependent on end-point concentration for a set of samples which vary in toxicity. Control limits can be adjusted for this effect. A regression line gives the expected range at a given end-point concentration. A control limit is calculated as 3.267 x expected range (King 1984; ASTM 1986) as presented in Figure 3.

Range-Type Control Charts for Reference Toxicants

When the end-point is expected to be relatively constant, as in a reference toxicant test, there should be no relationship between duplicate range and end-point concentration, and ranges can be charted in temporal sequence, rather than against end-point concentration (as in Figure 4). This approach facilitates early detection of developing trends in test precision. The mean range (R) provides the central line of the control chart. Warning and Controls limits are calculated as 2.456 x R and 3.267 x R, respectively. (King 1984; ASTM 1986).

USE OF MORE THAN ONE REFERENCE TOXICANT

Some proponents of reference toxicant testing recommend that laboratories employ two reference toxicants that represent diverse modes or sites of toxic action (frequently an organic plus an inorganic chemical). The rationale is that abnormal test organisms may not show a significantly different test response to one chemical, but that tests using a different chemical may detect the abnormal condition. This seems to be of greatest concern relative to effluent testing, where abnormal organisms may respond unusually to a particular effluent constituent while responding normally to a reference toxicant with a different mode of action.

The database in the literature is inadequate to assume that any given reference toxicant will be particularly effective in detecting abnormal organisms. The few published studies have demonstrated that even where tests differentiated between groups of fish, the relative change from the initial end-point was less than 75% (Dorn and Rodgers 1989; Alexander and Clarke 1978; Adelman and Smith 1976; Hansen 1979). Therefore, with respect to a control chart, a change in organism health may not result in an outlying point.

In addition, there are substantial sources of variability in both pure chemical and effluent tests besides organism health. For example, Dorn *et al.* (1987) reported that the coefficients of variation associated with preparing reference toxicant test solutions were 15 to 136% in

FIGURE 3: RANGE - TYPE CONTROL CHART FOR EFFLUENTS

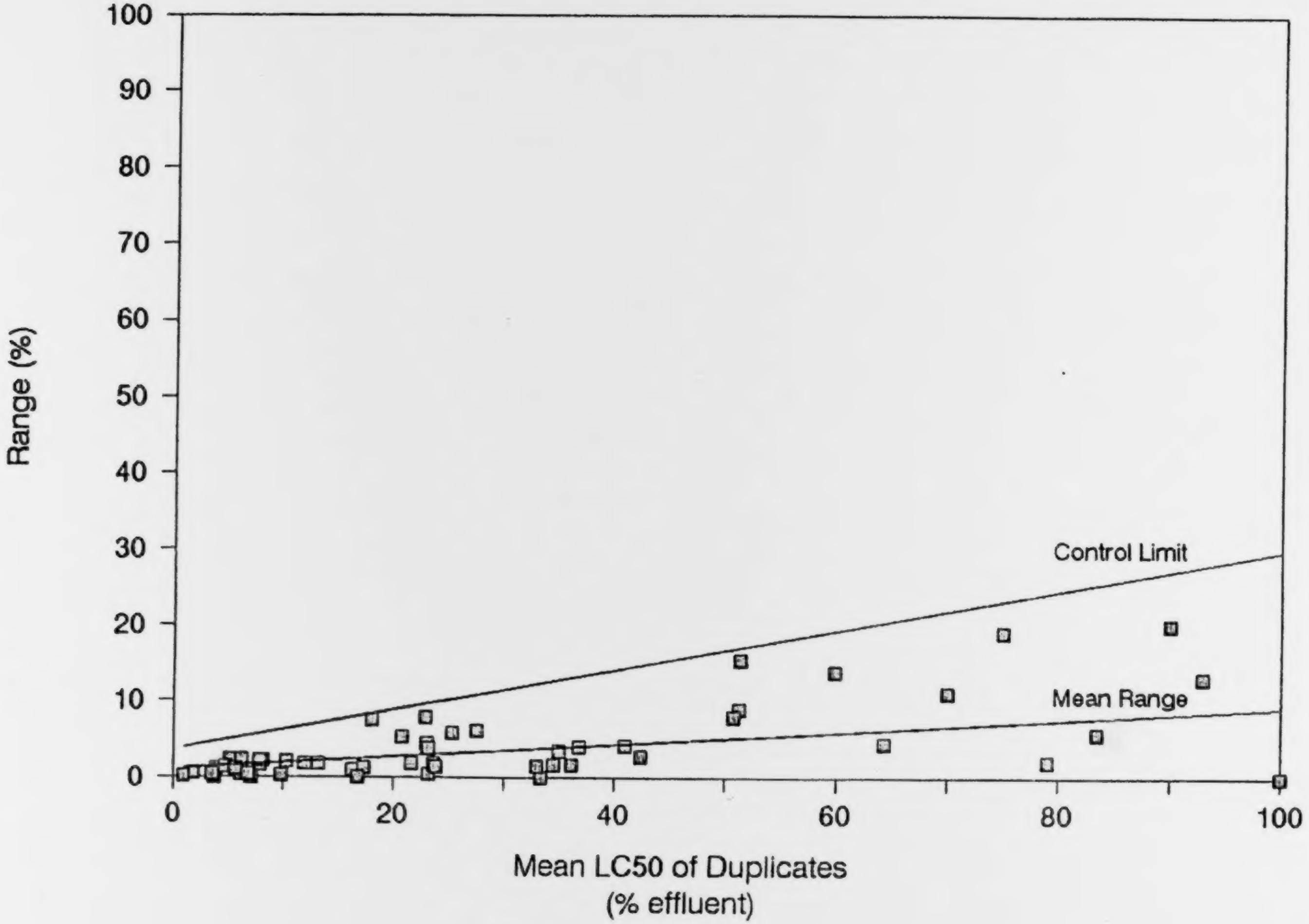
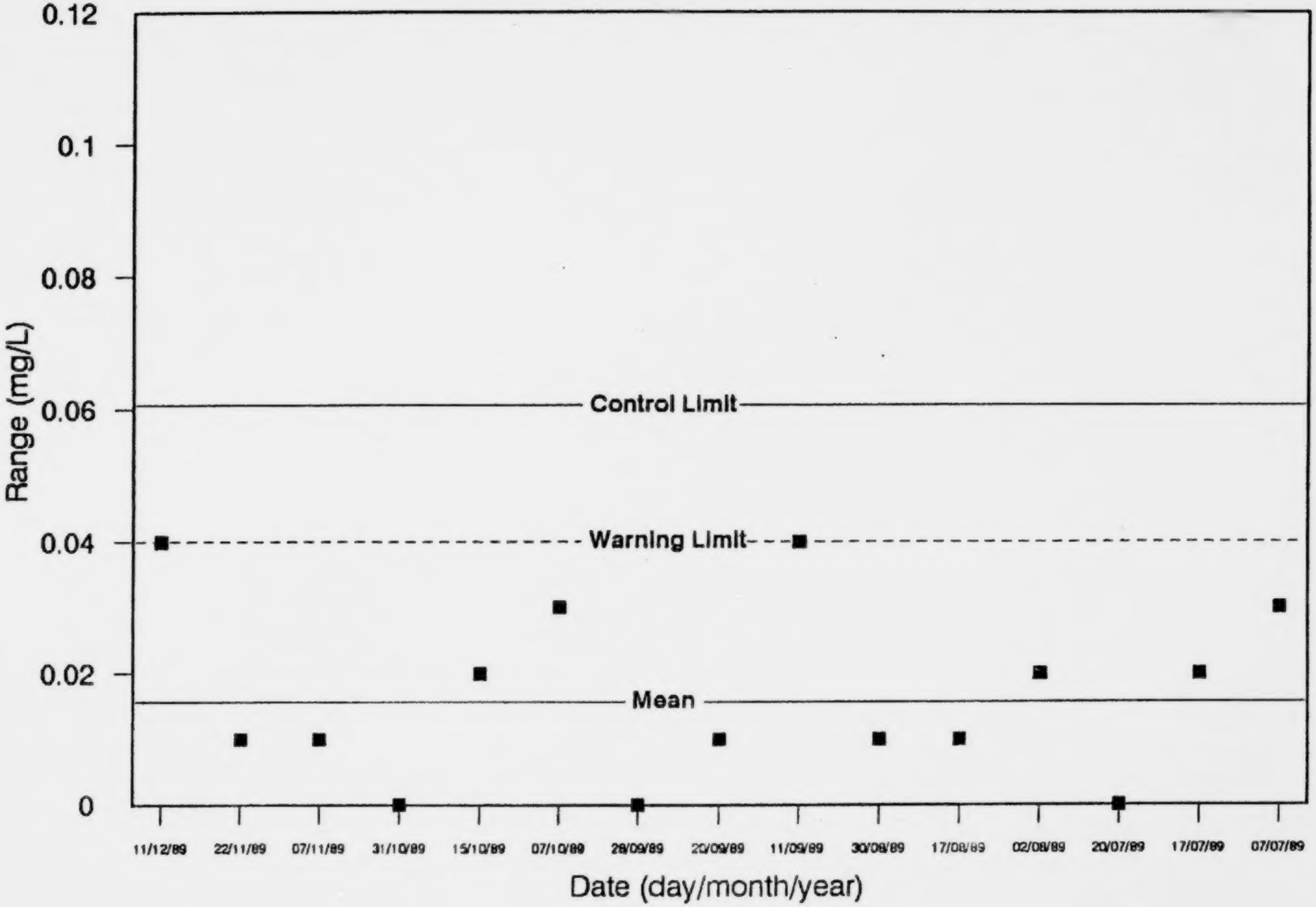


FIGURE 4: RANGE - TYPE CONTROL CHART FOR REFERENCE TOXICANTS



two laboratories. However, an outlier caused by this source of variability would not be directly relevant to effluent tests due to differences in solution preparation techniques between the two test types.

It is, therefore, inappropriate to put too much weight on a reference toxicant test to identify the specific factors that may influence an effluent test result; rather, the reference toxicant test results provide one general indicator of performance in a given test system. Rigorous attention should also be directed to other QA/QC activities, such as blank controls during tests, test replication, ring-testing, monitoring the health/growth/reproduction of test organism stocks and cultures. Records of all QA/QC activities should be reviewed whenever an effluent or a reference toxicant test appears unusual in order to identify a possible cause. It is therefore concluded that there is insufficient justification at this time to recommend that a second reference toxicant is likely to provide more information than one.

REFERENCE TOXICANTS IN SEDIMENT TESTS

Sediments have been well-recognized as a sink and/or source of persistent toxic chemicals (Zarba 1989; Ross & Henebry, Karickhoff & Morris 1985). Sediment contaminants exhibit complex interactions with physical/chemical properties of the sediment (Karickhoff *et al.* 1979). Organic carbon content, for example, is directly related to contaminant sorption, particularly for hydrophobic chemicals. This reduces toxicity since the chemical is less available for uptake by biota. Similarly, contaminant sorption tends to decrease with increased particle size, resulting in increased bioavailability and toxicity (Karickhoff *et al.* 1979).

Sediment toxicity tests have been developed to measure the biological impact of sediment-associated contaminants (e.g., Swartz *et al.* 1985; Nebeker *et al.* 1984; U.S. EPA/U.S. ACOE 1977). A variety of approaches have been used in sediment assays depending on the objectives of the study, e.g.:

- spiking - contaminant(s) is/are mixed into sediment or added to overlying water; duration of mixing stage important;
- mixing - combination of different sediment layers;
- seiving - to achieve homogeneity of particle size;
- dilutions - to establish dose-response;
- elutriate - determination of toxicity of water-soluble phase;
- sterilization - inhibition of biological activity.

In addition, some tests may combine more than one of the above approaches and may involve the use of either standardized or natural sediments.

Spiking control sediments with a reference toxicant to monitor test precision is conceptually analogous to using reference toxicants in aqueous toxicity tests using laboratory dilution water. In sediment testing, however, a control typically consists of a "clean" sediment (lacking the contaminants of the test sediment) that has similar characteristics to the test sediment (e.g., organic carbon content, particle size distribution). In this, sediment tests often differ from aquatic tests since control medium characteristics differ between tests, whereas dilution water controls in aquatic tests are relatively consistent over time. Therefore, if different control sediments were spiked with a reference toxicant, it is unlikely that the sources of variability that the laboratory wishes to monitor and control (e.g., organism health, technician performance, etc.) could be separated from the variability associated with differences between each test system (e.g., sediment characteristics).

To partially address this problem, water-borne reference toxicant exposures of sediment (benthic) test organisms have been recommended as a QC measure associated with sediment tests (Tetra Tech Inc. and E.V.S. Consultants Inc. 1986). Test duration may need to be restricted (e.g., four rather than ten days) since biota may become stressed by lack of substrate (R. Swartz, pers. comm.), and water column tests are probably inappropriate for biota that are highly dependent on substrate (e.g., chironomids). While water column tests provide a measure of variability associated with some aspects of sediment tests (e.g., organism health), they do not provide an overall measure of the reproducibility of a given sediment toxicity test method in a given laboratory.

A suggested additional/alternative QC practice to water column tests is to use a standardized sediment spiked with reference toxicant to monitor test precision over time. The test method employed should be the same (or as representative as possible) as the method typically used by the laboratory. Separate reference toxicant tests should be performed for each distinct test method-test species combination. The sediment spiking method should be well-defined to reduce the error associated with contaminant bioavailability. It is critical for system control purposes that the characteristics of the standardized sediment remain consistent over time and are reproducible.

The field of sediment toxicity testing is very new relative to aquatic testing (particularly in the area of QA/QC), and considerable work will be required before the most effective QA/QC approaches are identified. Meanwhile, it is suggested that the principles outlined previously for aqueous testing be adapted where possible to sediment tests.

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MANAGING TOXICITY DATA FOR REGULATORY PURPOSES

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ABSTRACT

The Aquatic Toxicity Unit evaluates large numbers of aquatic toxicity tests results submitted by industry to comply with Ontario's effluent monitoring regulations. We expect to have reviewed more than 20,000 bioassay test results within the next two years. After reviewing bench forms from most of the aquatic toxicity laboratories in the country, we developed an electronic database system for toxicity test data called TOXDATA. The Unit has four permanent staff and three student person years devoted to managing this data and performing the necessary audit tests. Since it is important to maintain integrity and validity of the data, therefore, everything required under regulations has had to be done according to written and agreed to protocols; all necessary information had to be included in the data reported; we have reported on it to all who need to see it; we have been checking it to see if it complies with the regulations. It is difficult to take any action without these prerequisites. Yet if we failed to put details in the test protocol that can be used later to dismiss the entire database as invalid or ambiguous, we will have wasted our entire effort. Having one and only one corporate database for toxicity test data that includes both detail and summary is essential to ensure the future of the data.

SUMMARY

Ontario evaluates large numbers of aquatic toxicity tests results submitted by industry to comply with effluent monitoring regulations. These regulations apply to all major dischargers in the province for one year. The Aquatic Toxicity Unit expects to have reviewed more than 20,000 bioassay test results within the next two years. Our experiences with this activity may be of some benefit to others in the same situation (eg. new regulatory requirements for toxicity testing in the Canadian Fisheries Act).

Approximately two years before promulgation of these regulations, the Aquatic Toxicity Unit began developing an electronic database system called TOXDATA. Its purpose was to deal with these test results and to handle our own data. We wrote it in DBase and compiled it in FoxBase. We realized that there would be continued requests for additional features, so we built the structure to fit as much information on each toxicity test as was logical. We still get requests for the program to provide accounting information, additional chemical parameters, more convenient entry features, more games, etc. We have added a few new features that met our original objectives but each change made during the regulation, no matter how small, tends to influence subsequent negotiations with industry. A stable program is much more important than satisfying everyone's specific request.

We designed TOXDATA for effluent samples but it can accommodate specific toxicants also. We designed it for acute lethality tests but it can be used for most chronic lethality tests. Generally, any test organism can be used although most of our data is for either rainbow trout or *Daphnia magna*. We expect to make few changes to the present version of TOXDATA except some that we recently negotiated with the federal environment department to satisfy the requirements of national protocols. TOXDATA will remain as the "front-end" data capture mechanism for our new effluent limits regulations. We are transferring the data corporately into an Oracle database which will allow us the flexibility we need for report writing and for linking to other data.

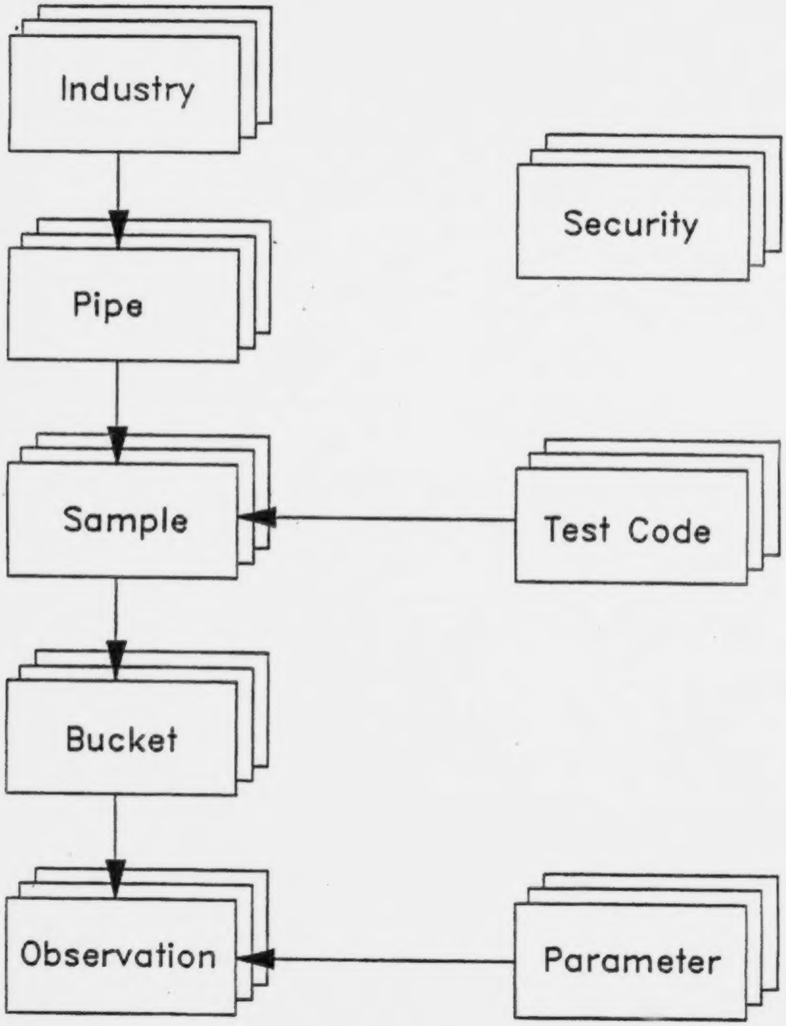
Industry
Security
Pipe
Sample
Test Code
Bucket
Observation
Parameter

Fig. 1. TOXDATA database structure

We developed TOXDATA after reviewing bench forms from most of the aquatic toxicity laboratories in the country. Its structure is a simple and logical set of linked tables (Fig. 1). The Discharger table contains information about the industry and its location including latitude and longitude. Linked to this through a unique discharger code, is the Pipe table that describes the various discharge points that may be at a given industrial site. The

Table 1. TOXDATA fields

DISCHARGER TABLE	TYPE	SIZE
DISCHARGER	NUM	10
INDUSTRY	CHR	35
INDALIAS	CHR	35
LOCATION	CHR	25
REGION	CHR	25
DISTRICT	CHR	25
SECTOR	CHR	50
INDXREF	CHR	10
RECWATDR	CHR	64
RECWATINDR	CHR	64
LAT	NUM	10
LONG	NUM	10
PROV	CHR	4
NOTES	MEM	10
SUMMARY	MEM	10

SECURITY TABLE		
PASSWORD	CHR	8
CLEARANCE	CHR	1
IDNAME	CHR	30

BUCKET TABLE		
SAMPLECODE	CHR	9
BUCKETCODE	CHR	1
CONCENTRAT	NUM	16
LT50	NUM	10
LT50RANUPP	NUM	10
LT50RANLOW	NUM	10
LT50CONUPP	NUM	10
LT50CONLOW	NUM	10

PIPE TABLE		
DISCHARGER	NUM	10
PIPE	NUM	4
PIPENAME	CHR	25
ANNUALFLOW	NUM	16

PARAMETER TABLE		
PARAMCODE	CHR	2
PARAMNAME	CHR	35
PARAMUNITS	CHR	25

SAMPLE TABLE		
SAMPLE	CHR	8
SAMPLEXREF	CHR	8
PIPE	NUM	3
DISCHARGER	NUM	10
LAB	CHR	10
SUBSTANCE	CHR	10
SMPLMETHOD	CHR	25
NAMECOLL	CHR	15
DATECOLL	DATE	8
DATEREC	DATE	8
DATESTART	DATE	8
TIME	NUM	4
SHIPMETHOD	CHR	25
STORAGE	CHR	15
TESTCODE	NUM	4
NOANIMALS	NUM	2
PREAERATED	LOG	1
AERATED	LOG	1
CALCMETH	CHR	25
LETHAL	LOG	1
LC50	NUM	11
LC50UPPER	NUM	11
LC50LOWER	NUM	11
CONCUNITS	CHR	10
NOTES	CHR	45
LC50INTLOW	NUM	11
LC50INTUPP	NUM	11
DILUTION	CHR	15
SLOPE	NUM	3

TESTCODE TABLE		
TESTNUMBER	NUM	4
FISHSPECIE	CHR	20
LIFESTAGE	CHR	20
FISHSOURCE	CHR	20
TESTVOLUME	NUM	7
TESTTYPE	CHR	10
FISHWEIGHT	NUM	5
FISHLENGTH	NUM	5

OBSERVATION TABLE		
BUCKETCODE	CHR	9
OBSERVATIO	CHR	23
OBSERVDATE	NUM	8
OBSERVTIME	NUM	4
PARAMCODE	NUM	2
VALUE	NUM	16

Discharger table also has a field for cross referencing Ontario's discharger codes with other systems. Linked to the Pipe table is the Sample table containing information about a specific sample including sampling and testing dates, test method used, and results. Linked to the Sample table is a Bucket table with information relating to a specific concentration. Linked to the Bucket table is an Observation table containing observations of mortality and simple chemistry done on each bioassay concentration at various times during the test. In addition, there are look up tables for describing toxicity test procedures, for chemical parameters, and for system security. Table 1 is a list of fields taken from the TOXDATA manual (Lee et al. 1989).

It is essential that everything required under regulations must be according to written and agreed to toxicity test protocols (eg. Craig *et al.* 1983, Poirier *et al.* 1988). For each requirement in the test procedure, all necessary information must be in the data reported, we have had to be prepared to report it to all who need to see it, and we have had to check it to see if it complies. It is difficult to take any action without these prerequisites. This seems obvious, but wait until the data starts rolling in! Thinking through the resource needs in advance makes the difference between success and failure. On the other hand, if we have failed to put a detail in the test protocol that someone can use later to dismiss the entire database as invalid or ambiguous, we will have wasted our entire effort. It would be a mistake to keep the detail in one place and the summary in another because one can almost guarantee that they will drift apart. Having one and only one corporate database is the only way to ensure the future of the data.

The Aquatic Toxicity Unit has assumed the responsibility of reviewing, maintaining and reporting on the toxicity data. The Ministry's regional offices receive Toxicity test results from industry in hard copy and on floppy disks. They then send them to us. Since there have been errors in these, we have had to let a contract to have them checked and printed. If there are problems, we advise the regional office to ask industry to resubmit. If there are no technical problems, a toxicologist on our staff reviews the test results against the requirements of the protocols. We also advise the regions of these problems. Though we perform this review normally within days of receiving the data, there is nearly three months between sampling and review. I doubt if this time could be shortened. We receive between 30 and 50 disks per week. We audit the industry self-monitoring with about 600 bioassays we do ourselves and another 800 through contract labs. We also produce data reports twice a year for each of nine industrial sectors. Table 2 summarizes the timing and size of each sector. Requirements for municipal sewage treatment plants are currently being revised.

The monitoring regulations require monthly both trout and *Daphnia* toxicity tests which may be quarterly under some circumstances. The manpower costs of this effort are not trivial. There are four permanent staff and three student person years devoted to the above tasks. In addition, three more staff and two students perform related tasks of developing future procedures and negotiating regulatory requirements. It was crucial for us to secure the necessary resources before beginning this regulatory effort.

Table 2. Summary of Ontario's Effluent Monitoring Regulations in terms of numbers of samples and schedule.

SECTOR	NO. OF SITES	NO. OF CONTROL POINTS	START OF SAMPLING
PETROLEUM	7	34	DEC, 1988
ORGANIC CHEM.	27	127	OCT, 1989
IRON AND STEEL	7	76	NOV, 1989
INORGANIC CHEM.	29	92	DEC, 1989
PULP AND PAPER	27	103	JAN, 1990
MINING	56	68	FEB, 1990
METAL CASTING	10	16	MAY, 1990
ELECTRIC POWER	24	282	JUN, 1990
INDUSTR. MINERALS	50	59	OCT, 1990
MUNICIPAL SEWAGE TREATMENT PLANTS	being revised		

REFERENCES

Craig, G., K. Flood, J. Lee and M. Thomson. 1983. Protocol to determine the acute lethality of liquid effluents to fish. Ontario Ministry of the Environment, 9pp.

Lee, J.T., R.D. Dale, and G.F. Westlake. 1989. Toxicity relational database system TOXDATA version 2.0 User's Manual. Ontario Ministry of the Environment. ISBN: 0-7729-5463-1. 42pp.

Poirier, D.G., G.F. Westlake, and S.G. Abernethy. 1988. *Daphnia magna* acute lethality toxicity test protocol. Ontario Ministry of the Environment. ISBN: 0-7729-3798-2. 29pp.

REFINERY EFFLUENT BIOMONITORING

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ABSTRACT

In 1978, Sprague et al. conducted an extensive examination of refinery effluent. The study was supported by the Petroleum Association for the protection of the Canadian Environment (PACE) and by the Canadian Government. A biomonitoring project which complements and updates the Sprague work is currently under way. This paper examines preliminary results of tests conducted on two refinery effluents using eight different organisms and 24 end points. Full life cycle tests using *Medaka* are being conducted. The plan includes methodology for linking earlier work with present protocols. For many years, the Lambton Industrial Society (LIS) has supported effects monitoring in the St. Clair River. The approaches used have often been innovative. This includes programs such as a 2 year flow through rainbow trout study (growth, flesh analysis and histopathology), egg hatching, the use of in-situ spot tail shiners, and flow through *Daphnia* chronic tests. Comments on regulations and biomonitoring, from an industrial perspective will be made.

SUMMARY: CPPI PLAN

In 1975 and '76, Sprague and Associates conducted an experiment funded by PACE (now CPPI) and by the Canadian Government. Treated refinery effluent from BP Refinery, Oakville, Ontario (now Petro-Canada) was tested. CCIW in Burlington supplied the laboratory services.

Some of the findings from that 300 page document are summarized as follows:

Table 1. Results from numerous samples of effluent taken over a 2 year period in 1975 and '76.

Test	response	comment
Rainbow trout	lethality	64% of samples non-lethal
	growth	EC50 approx. 10%
	avoidance	no effect to 30%
	respiration	50-100%
Flagfish	lethality	
	growth	} similar to trout
	respiration	
D. pulex	lethality	LC50 76%
	reproduction	approx. 0.5% eff. "safe"

Conclusions:...sublethal effects could not be correlated to chemical characteristics of the effluent. *D. pulex* was 2.5 times more sensitive than trout and useful as a screening test. Smaller fish were preferable for tests.

The purpose of this intensive study was to "extend knowledge of the effects...of treated refinery effluent beyond the bounds covered by current Canadian government regulations and guidelines." (Sprague, 1978).

The Canadian Petroleum Products Institute (CPPI), "as part of continued scientific studies into the quality of refinery effluents" (Chapman, 1989), recognized the need to update the earlier work.

Table 2. Refinery effluent biomonitoring plan summary:

- update and complement the Sprague work.
- eight different organisms: Rainbow trout, fathead minnows, Medaka, D. magna, D. pulex, Ceriodaphnia, Selenastrum, Microtox)
- 24 different toxicity end points
- objectives: to document changes in effluent quality,
  to determine any potential for chronic toxicity,
  comparison of protocols,
  publish the results for further comparative work,
  tiered plan.

This biomonitoring plan included a comparison of various protocols. A typical comparison is included in the appendix: (comparison of *Daphnia* acute lethality protocols).

PRELIMINARY RESULTS

The data presented here was conducted by EVS Consultants, and by Pollutech Environmental (Moran, 1990): EVS prepared the biomonitoring plan for the refinery work and Pollutech is currently conducting the biomonitoring work for the CPPI refinery study. Recognize that only a summary of the raw data is presented here. The final study will be released as as a CPPI publication next year, once the data has been reviewed, analyzed and compared against the previous work.

Table 3. NOVA Petrochemicals Inc. (Corunna Operations). Effluent:

1. *D. magna, pulex* and Rainbow Trout: no toxicity or immobility.
2. *Ceriodaphnia dubia* 7 day reproductive: no acute effect. Enhanced growth of adults in 100% effluent. The effluent has numerous carnivorous copepods, which made counting difficult (missing neonates?)
3. *Selenastrum capricornutum*: numerous algae, diatoms and planktiovores present in effluent. EC50..10%?

4. Microtox... no toxicity in any sample.
5. Fathead minnow egg teratogenicity and larval survival. No mortality at egg stage. Mortality was observed at hatch out: fins missing due to carnivorous copepods (>10% concentration).
6. Fathead minnow larval survival and growth test: Control weight 0.3 mg., 100% effluent 0.26 mg., and intake 0.23 mg. Survival was better in 100% effluent due to the presence of food.
7. *D. magna* 21 day reproductive: enhanced growth in effluent.
8. *D.pulex* 21 reproductive: enhanced growth in effluent.
9. Medaka *(Orizias latipes)*: toxicity observed down to 10%. Other observations: fungi, parasitism of eggs, mid concentrations affected first.

Table 4. Petro-Canada (Oakville) effluent:

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1. *D. magna, pulex* and Rainbow Trout: no toxicity or immobility.
2. *Ceriodaphnia dubia* 7 day reproductive: effects at approx. 10-25%.
3. *Selenastrum capricornutum*: effects at >10%.
4. Microtox... no toxicity in any sample.
5. Fathead minnow egg teratogenicity and larval survival: * teratogenic effects observed at approximately 56%.
6. Fathead minnow larval survival and growth test: * effects in 75-100%
7. *D. magna* 21 day reproductive: * small broods in 100% effluent
8. *D.pulex* 21 reproductive: * neonate production at about 10% effluent
9. Medaka *(Orizias latipes)*:* all eggs hatched, with no abnormalities.

* tests in progress

SUMMARY: LAMBTON INDUSTRIAL SOCIETY BIOMONITORING

The Lambton Industrial Society is a non-profit industry funded environmental cooperative. The purpose of the Society is to promote and foster, through joint and individual effort by the member companies, the protection of the environment (air, land, and water) consistent with the standards set by government regulation and good corporate citizenship.

Members are professional environmental staff of local Lambton County (Sarnia, Ontario) industries:

Cabot Canada Limited
Dow Chemical Canada Limited
Ethyl Canada Inc.
Imperial Oil Limited
Novacor Chemicals Limited
NOVA Pterochemicals Inc (Sarnia)
Shell Canada Products Limited
Laidllaw Environmental Services Limited

ICI Canada Limited
Du Pont Canada Inc.
Fiberglas Canada Inc.
Lambton Thermal Generating Station
NOVA Petrochemicals Inc. (Corunna)
Polysar Rubber Corporation
Sunoco Inc.
Partec Insulations Limited*

* Associate member

The LIS has often played a leadership role in environmental effects monitoring, with a 38 year history of monitoring and numerous innovative programs to their credit. In biomonitoring, the LIS has commissioned 40 reports at a cost of approximately $1 million. These reports are unpublished, but are in the public domain and are available for review at the LIS library. The LIS does not conduct pure research. The "research" we conduct is one of applying good science to find out more about real concerns. One of the earliest biomonitoring work conducted by the society was a benthic survey of the St. Clair River.(Beak, 1973). In 1975, taste and odour testing of fish, using 3 different methods were conducted:

> 1: fish were maintained in a number of stations located along the shore, 2: by using caged fish stationed in the river, and
>
> 3: by catching indigenous fish from lake Huron, lake St. Clair and the river.

Fish growth studies were also conducted. During this early time period growth was poor and occasional fish kills in the river occurred. By 1979, the methodologies had been well tuned with a sound statistical procedure developed. Work had begun on the identification of volatile organics in fish and water. In the 80's, fish taste and odour testing continued... fish flesh was analyzed for 16 organic compounds using gc/ms techniques and the findings compared to the taste and odour results. It was found for example that compounds like benzene washed out of the fish flesh after 10 days exposure to clean water. (Pollutech, 1982).

Monitoring programs shifted from acute to subtle effects and to trace substances. Fish growth studies were restarted on a year round basis. Fish flesh taste and odour monitoring was discontinued, but fish pathology, spawning potential, and egg hatching was instituted in '87 to '89. During this time, a two year continuous (flow through) test was completed. Rainbow trout were continuously exposed to river water, and fish growth, pathology and fish flesh analysis were conducted. This year a number of studies have been conducted, and both results and methods are curently being assessed.

1990 Biomonitoring Activities

- Flow through evaluations of *Daphnia magna & pulex*
  - number of days to first brood
  - number of neonates/brood
- Rainbow trout egg hatchability
- Sediment toxicity: 7 locations, chemistry, +ve and -ve controls.
  - amphipods
  - D. magna
  - microtox
- On site *D. magna* and *pulex* river water flow through studies
  - with and without feed, filtered and unfiltered.
- Fathead minnow flow through river water testing.
  - larval growth, fecundity vs lab cultured.

Biomonitoring activities for 1991 are now in the approval stage. Results for the 1990 program will be available for public review in the spring of '91.

DISCUSSION AND COMMENTS

The information presented here demonstrates that the refining industry is proactive and is attempting to understand both the quality of its effluent and the potential impact of its operations on receiving water quality.

The proactive biomonitoring being conducted by the LIS and by CPPI will go a large step towards satisfying and understanding the new Environment Canada "Guidelines for Environmental Effects Monitoring...".

Governments tend to over regulate, (NCPA, 1989). Industry needs clearly understood, practical, fair and relevant biomonitoring regulations. The major objective is the protection of the environment. "Government extoles the virtue of dialogue in the regulatory agenda" (Alliston, 1988). The refining industry fully agrees with a cooperative approach and with dialogue. Our industry a few comments to consider when addressing regulations:

* A policy on water discharges is needed.
* Currently, we tend to focus on end-of-pipe, rather than assessing the benefits of a particular abatement strategy.
* Government, in consultation with stakeholders, can and should provide a leadership role for ensuring that we work on the right problems with the scarce resources available.
* In the development of the CPPI plan, it became evident that various protocols have been used for a particular test. The use of a rigorously tested and reviewed protocol is essential for regulatory compliance testing. The test should include QA/QC and positive controls.
* A thorough, peer reviewed guidance document should be a part of a compliance regulation. Such a document is essential for the public and for the discharger to understand a complex subject such as biomonitoring.

Examination of data from various sources is critical in understanding a problem, formulating plans for remediation, or in solving a problem. The LIS has relied upon independent, innovative and adaptable techniques to monitor the quality of the river.

Don't be afraid to look.

The further we go with biomonitoring and with regulations, and the more the public becomes aware of various environmental issues, then the more sense industrial organizations such as the LIS and CPPI make sense. We can monitor/assess the environment piecemeal or look at its overall health. Industrial organizations have a larger resource base than individual companies, can look at real effects, and be less driven by the political process. Cooperation and the sharing of information is a critical activity. Only then may we all better understand the problems, prioritize, and then tackle the right ones together. We live here too.

Table 5. Comparison of recent *Daphnia* acute lethality protocols.

Parameter	Peltier and Weber, 1985 (U.S. EPA)	E.P. Draft, 1989b	Poirer et al., 1988 (Ontario M.O.E.)	ASTM, 1984
Test Organism**	*Daphnia magna/pulex*	*Daphnia magna/pulex*	*Daphnia magna*	*Daphnia magna/pulex*
CULTURE PROTOCOL				
Health requirements	No ephippia or ♂'s produced	<25% incidence of ephippia. Short time to first brood (<14 d). Large broods (>20 for *D. magna*). Sensitivity to ref. tox.	No ephippia. Many young and few adults. Short time to first brood (<18 d). Large broods (>30).	NS
Age/life stage/size	All	NS	NS	All
Temperature (°C)	18-26 (20 best)	20 ± 2	20 ± 1	NS
Light:				
• quality	NS	"Cold white" fluorescent. CRI ≥ 90	"Cool white" fluorescent. CRI ≥ 90	NS
• intensity (lux)	540-1080	800 at surface	800 at surface	NS
• photoperiod	16 h/8 h (or longer light)	16 h/8 h	16 h/8 h	NS
• dimming period (min)	NS	NS	NS	NS
Culture chamber:				
• material	Glass	Non-toxic	All glass/Nalgene	NS
• design	Beaker	NS	NS	NS
• size	NS	NS	> 4 L	NS
Culture medium:				
• requirements	hardness 160-180 mg/L *D. magna* hardness 80-90 mg/L *D. pulex* pH 7.0-8.6	GW, SW, DCW, RC hardness 40-48 mg/L *D. pulex* hardness 80-100 mg/L *D. magna*	Sufficient to maintain culture. DCW; Cl_2 undetectable by amperimetric titration. Hardness 120-250 mg/L; pH 6.5-8.5.	Contaminant-free

Parameter	Peltier and Weber, 1985 (U.S. EPA)	E.P. Draft, 1989b	Poirer et al., 1988 (Ontario M.O.E.)	ASTM, 1984
Type of holding design	SR (~100% weekly)	SR (~100% weekly)	SR (25% weekly)	NS
Loading density	30/vessel or 10/L	8/L (≤ 10/L for broodstock)	(10/L for Broodstock)	NS
Aeration:				
• quality	Oil-free	Filtered, oil-free	Filtered, oil-free	NS
• requirements	D.O. must be >3 mg/L (pref. 6 mg/L)	≥ 60% saturated	NS	NS
Feeding:				
• diet formulation	Trout chow, alfalfa and yeast or *Selanastrum*	Several algae and yeast	Two algae	NS
• regime/amount	3X/week	as req'd to maintain metabolism	2 X/week	NS
Monitoring:				
• water quality	NS	D.O., hardness. Within 20% of control/dilution water parameters	NS	NS
ACCLIMATION PROTOCOL				
Holding at test temperature	NS	>7 d	NS	NS
Health criteria for testing	NS	<25% incidence of ephippia 7 d prior	NS	NS
SAMPLE HANDLING PROTOCOL				
Containers	NS	Clean, inert, filled and sealed	Clean, inert or lined, filled and sealed	Covered, unsealed
Transportation	"On ice"	<8°C (>0°C)	NS	Do not agitate violently
Time to test (unrefrig.)	NS	NS	NS	<24 h (or demonstrate tox. @ t>24 h >50% of tox. @ t<24 h

Parameter	Peltier and Weber, 1985 (U.S. EPA)	E.P. Draft, 1989b	Poirer et al., 1988 (Ontario M.O.E.)	ASTM, 1984
Refrig. temperature (°C)**	4	4 ± 2	5-15	4
Time to test (refrig.)	≤ 72 h (pref. <24 h)	≤5 d	<5 d	NS
Resuspension of sample	NS	NS	Before testing	Before testing
TESTING PROTOCOL				
Source of organisms	In-house brood culture	In-house brood culture	In-house brood culture	In-house brood culture
Age/stage/size	1-24 h neonates	< 24 h neonates	< 24 h neonates	< 24 h neonates
Duration of test (h)	24 for screening;48 for definitive	48	48	48
Temperature (°C)	20 ± 2	20 ± 2	NS (20 ± 2 for culture)	20 ± 2
Light:				
• quality	Ambient lab illumination	"Cold white" fluorescent. CRI≥90	NS	Wide spectrum fluorescent (CRI≥90)
• intensity (lux)	540-1080	800 at surface	NS	800
• photoperiod (lt/dk)	8 h/16 h to 16 h/8 h	16 h/8 h	NS	16 h/8 h
• dimming period (min)	NS	NS	NS	NS
• synchrony with culture photoperiod	NS	Yes	NS	NS
Test chamber:				
• material	Inert	Glass/borosilicate glass	Glass	Borosilicate glass
• design	Beaker	Beakers	Test tube (low surface area/volume)	Beaker
• volume	100 mL	150-250 mL	~75 mL (any size)	250 mL
• covered	Yes; with an opaque cover	Yes	NS	NS
Test solution volume	50 mL	NS	50 mL	200 mL

Parameter	Peltier and Weber, 1985 (U.S. EPA)	E.P. Draft, 1989b	Poirer et al., 1988 (Ontario M.O.E.)	ASTM, 1984
Type of test**	Pref. SR or FT	S (SR/FT if volatile)	S	S
Dilution/control water:	Pref. URW. Other	Depends on intent. GW, SW, DCW, RC, URW	Same water as used to maintain culture.	Pref. URW of stable quality, or RC
• requirements/ description	hard water for *D. magna* moderately hard water for *D. pulex*	D.O.≥90% saturated pH 6-9	DCW; Cl_2 undetectable. Hardness 120-250 mL; pH 6.5-8.5; Aerated overnight	Neonates must be able to survive in it for 48 h without food
Aeration:	Only if necessary	If necessary	No, unless necessary/useful	Of dilution water prior to use
• quality	Oil-free	Filtered, oil-free	Filtered, oil-free	NS
• quantity	<100 bubbles/min.	7.5 mL/min/L @ start for <120 min	NS	"Intensely"
• requirements	D.O. >40% saturated	D.O.>60%(5.5 mg/L)	NS	NS
# organisms/chamber	10	NS	3/50 mL	5
# organisms/treatment**	≥20	≥10	>10	NS
Stocking density**	200/L	<67/L	<67/L	25/L
Transfer/Handling	Pipette; gentle and quick	Subsurface gentle pipetting	Subsurface gentle pipetting	Subsurface large bore pipetting
Feeding	No	No	NS	NS
Test concentrations	geo. series + control	5 (log. series) + control	5 (log. series) + control	≥ 5 (geo. series) + control
Replication	2	If desired, 2,3 or more	≥4	≥ 4
Randomization	NS	Chambers and organisms	NS	Chambers and organisms
Reference toxicant	SDS,NaPCP,$CdCl_2$	Phenol, potassium dichromate	NS	NS

Parameter	Peltier and Weber, 1985 (U.S. EPA)	E.P. Draft, 1989b	Poirer et al., 1988 (Ontario M.O.E.)	ASTM, 1984
Monitoring:				
• parameters	D.O.	Temp, pH, D.O. (conductance, hardness, residual Cl_2 + visual)	D.O., pH, hardness, conductance	Conductance, hardness, alkalinity, pH, temp., D.O., susp. solids
• times	NS	0,48 h (minimum)	0,48 h (minimum)	0, 48 h
Observations:				
• death	No movement on gentle prodding	No movement & no heartbeat	No heartbeat	Cessation of movement, no response to tap on beaker
• abnormal behaviour	NS	Immobility, lethargy, floating	Immobility, floating, circling	Immobility, floaters
• times	NS	0,1,4,24,48 h	1,2,4,24,48 h	NS
Acceptance criteria	LC50 for ref. tox. in range of normal values	<10% control mortality	<10% control mortality <100% mortality at lowest conc. above 0%	<10% control mortality
End points	LC50	24,48 h LC50. NOEC	48 h LC50/EC50	48 h LC50/EC50

CRI = Colour Rendering Index.
GW = Ground Water.
SW = Surface Water.
DCW = Dechlorinated City Water.
RC = Reconstituted Water.
URW = Upstream Receiving Water.
SR = Static Renewal/Replacement.
S = Static.
FT = Flow Through.
D.O. = Dissolved Oxygen.
LC50 = Median Lethal Concentration.
NOEC = No Observed Effect Concentration.
NS = Not Specified.
Ref. tox. = Reference toxicant.
log. = logarithmic
geo. = geometric
SDS = Sodium Dodecylsulphate
NaPCP = Sodium Pentachlorophenate
** = Substantial differences in parameters, which will impact test design/results.

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ACUTE AND CHRONIC TOXICITY EVALUATION OF ONTARIO STP EFFLUENTS.

G.R. Craig, H.M. Monteith, and P.L. Orr. Beak Consultants Ltd., 14 Abacus Rd., Brampton, Ont. Canada (416-458-4044).

Four composite and up to six grab effluent samples were collected from each of ten Ontario sewage treatment plants (STPs) under both summer and winter operating conditions. Acute lethality (rainbow trout and Daphnia magna) and chronic (fathead minnow and Ceriodaphnia dubia) tests were conducted. All samples were analyzed for conventional parameters, including total residual chlorine (TRC) and ammonia, as well as metals. Organics analyses were performed on selected samples.

The majority of STP effluents were only marginally lethal (most LC50s > 50%), but chronic effects were observed at concentrations as low as 14% effluent. Species sensitivity ranking was not consistent among samples. No significant difference was observed between the toxicity of grab vs. composite samples, but winter samples were more toxic than summer samples. TRC and un-ionized ammonia concentrations accounted for much, but not all, observed toxicity.

STPs categorized as having a low industrial loading produced effluents which were significantly less toxic to rainbow trout and C. dubia than those with high loading. No statistically significant differences in acute or chronic toxicity was associated with plant type.

CONSEQUENCES OF EPISODIC EXPOSURE OF CHUM SALMON EGGS/ALEVINS TO EXPECTED A-J MINE TAILINGS POND CONCENTRATIONS OF Ag, Cd, Cu, Pb AND Zn

(SUMMARY)

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ABSTRACT

CONSEQUENCES OF EPISODIC EXPOSURE OF CHUM SALMON EGGS/ALEVINS TO EXPECTED A-J MINE TAILINGS POND CONCENTRATIONS OF Ag, Cd, Cu, Pb AND Zn. J. W. Buell, Buell & Associates, Rt 3 Box 706, Beaverton, OR, USA (503-649-9205).

The preferred tailings disposal alternative for the A-J Mine, Juneau, AK, would episodically interrupt the water supply to a chum salmon hatchery, requiring the use of tailings pond water for egg/alevin incubation. A bioassay using five metals (Ag, Cd, Cu, Pb and Zn) produced excess mortality of experimental eggs/alevins over controls of 0.4% (98.7% experimental survival, 99.1% control survival), well within tolerable limits. Body burden and body compartment analyses tracked metals uptake during exposure and depuration during an extended (8-wk) salt water challenge. Long term increases in body burdens of Cd, Cu, Pb, and Zn in both experimental and control fish during salt water challenge were traced to the food supply. Survival during the 8-wk salt water challenge was much greater for the experimental group (98.8%) than for the control group (91.1%). Possible explanations are explored. It is concluded that the preferred tailings disposal alternative and continued hatchery operation can be compatible.

INTRODUCTION

The preferred alternative for A-J mine tailings disposal is construction of a tailings reservoir behind a dam at Sheep Creek stream mile 1.1. Substantially all Sheep Creek stream flow would be interrupted at this point, with water passing down a pipe to a marine outfall. The remaining watershed downstream of the dam site would continue to supply water to Sheep Creek in accordance with its size, elevation, aspect, etc.

The Douglas Island Pink and Chum (DIPAC) Sheep Creek Hatchery is located at the mouth of Sheep Creek and relies on the creek for its continuous water supply needs. As a result of changing operations, the water supply needs for the Sheep Creek Hatchery are evolving to about 2 cfs from early August through about mid-March, with 9-10 cfs needed in August and early September for adult fish attraction until all spawning activities are taken over by the Gasteneau facility. Although a hydrological analysis has shown that the lower Sheep Creek watershed after dam construction will continue to supply sufficient water to the hatchery most of the time, natural flow is expected to be insufficient to meet incubation needs during cold "snaps", which typically occur once or twice a year and may last up to two weeks.

One alternative for supplying water to the Sheep Creek Hatchery during periods of natural flow insufficiency is to transmit supplemental water from the tailings reservoir, either through a pipe or by release at the dam site. Pilot milling studies and modeling of expected tailings water constituents suggest that five heavy metals, silver, cadmium, copper, lead and zinc, alone or in combination, have some potential for interfering with the hatchery operations It was also determined, however, that if the hardness of the hatchery water were to be increased to a level which is ideal for incubating pink and chum salmon eggs and alevins (about 300 mg/l as $CaCO_3$), the probability of these five metals having any toxic effect would be reduced. In order to test this hypothesis, a bioassay was conducted at the Sheep Creek Hatchery using all five metals of concern and mimicing as closely as possible the conditions under which tailings reservoir water would actually be used to supplement lower Sheep Creek water for hatchery purposes.

The bioassay was designed and conducted to answer precisely this question:

> "Will the episodic exposure of developing chum salmon eggs and embryos to tailings pond water as a supplemental incubation water source for use during cold-induced low natural stream flow periods adversely influence survival or development if incubation water hardness is adjusted to about 300 mg/l as $CaCO_3$ with seawater?"

According to the agreement between Echo Bay and DIPAC, a negative effect of episodic exposure to the five metals on incubating pink salmon eggs / alevins to the extent that it may jeopardize continued successful operation of the Sheep Creek Hatchery will be judged to have occurred if survival of the experimental group is significantly less than 95% of the survival of the control group, unless the overall survival of the experimental group is greater than 90%, which will be interpreted as "no significant effect"

METHODS

EPA has recognized the exponential inverse relationship between water hardness and toxicity in many cases by promulgating water quality criteria which are often hardness-dependent. EPA chronic criteria for the five metals being used in the Sheep Creek Hatchery bioassay are plotted on Figure 1 to illustrate this phenomenon. Conversations with Dr. J. Helle (NMFS, Auke Bay) confirmed that elevating salinity to about 2 parts per thousand (6% seawater) provides ideal incubation conditions for pink and chum salmon eggs and alevins. This increase in salinity through seawater augmentation would result in a water hardness of 300 mg/l as $CaCO_3$. The combination of ideal salinity conditions and significant reduction of metals toxicities led to the selection of 6% seawater (hardness = 300 mg/l as $CaCO_3$) as the appropriate augmentation rate.

The experimental protocol used in this bioassay involved the episodic exposure of incubating chum salmon eggs / alevins to concentrations of the five heavy metals of concern at or above their expected concentrations in tailings reservoir water:

Metal	Test concentration
Silver	4 ppb
Cadmium	2 ppb
Copper	20 ppb
Lead	20 ppb
Zinc	20 ppb

These metals concentrations reflect expected reservoir concentrations taking into account only dilution with inflow from the surrounding drainage basin and some chemical equilibration and do not reflect complexation, adsorption onto particulates and precipitation out of the water column. All of these processes, which are known to occur under circumstances like those which will exist in the proposed reservoir, will reduce the actual concentrations of metals in water exiting the reservoir, probably quite significantly. In this respect, this bioassay is "worst case" and incorporates a conservative protocol.

The eggs used in this bioassay were obtained from adult chum salmon returning to the Sheep Creek Hatchery located at Thane (about 4.5 mi south of Juneau). Fish were spawned according to standard hatchery procedures on 06 August 1989. Eighty females

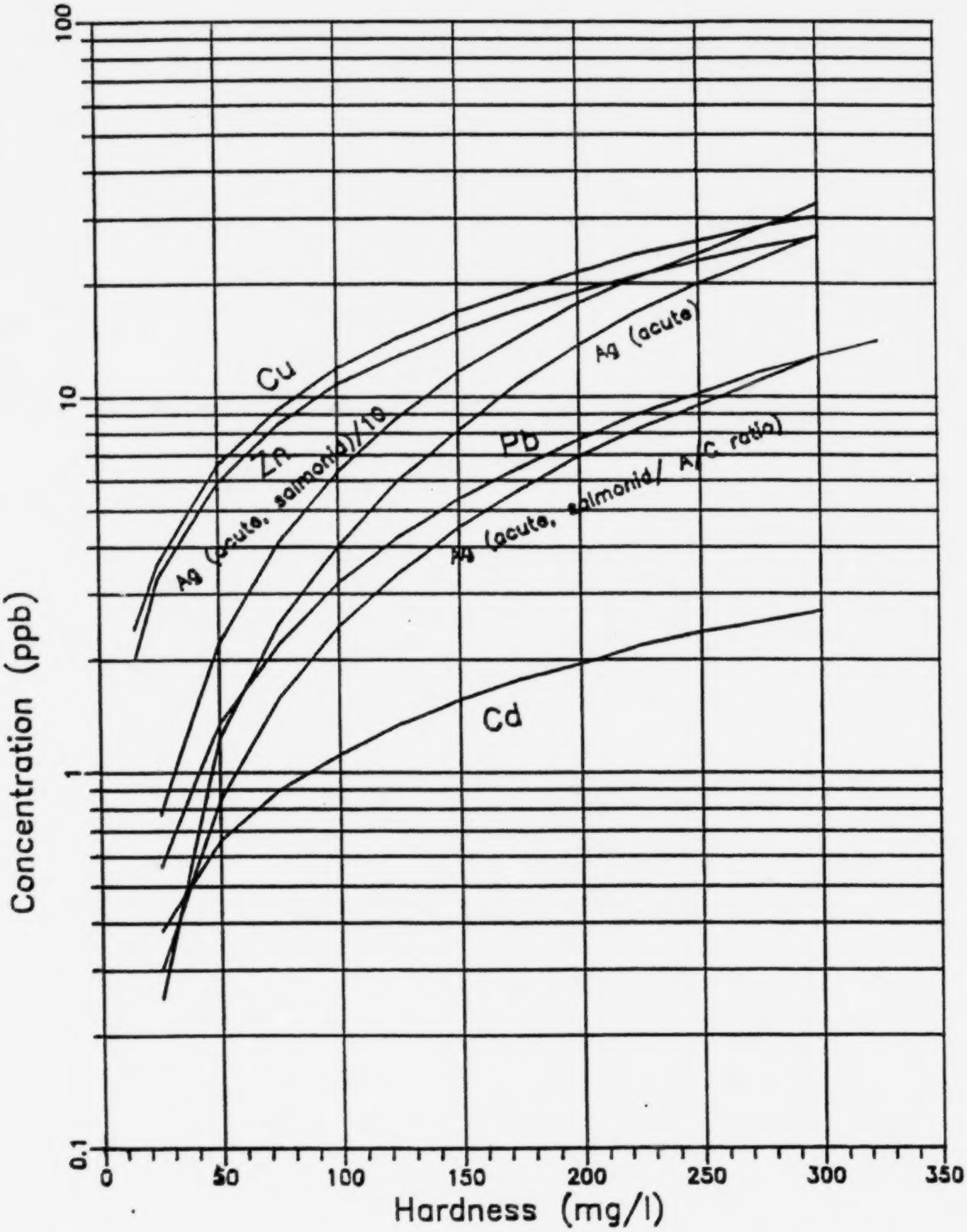


Figure 1. EPA chronic freshwater criteria

and about forty males were used, with milt and eggs pooled to ensure good fertilization. Eggs were placed in Heath trays to water harden and begin developing. On 03 October, after eyeing and prior to the first episodic exposure of the experimental group to heavy metals, dead and unfertilized eggs (about 1% per stack) were picked and viable eggs were redistributed among incubator trays to continue incubation.

The standard Heath trays were loaded with chum salmon eggs at normal densities (7,000 eggs / alevins per tray with odd lots in the last loaded tray of each stack). Two half-stacks were used as controls and two half-stacks were used for experimental eggs / alevins. Sheep Creek water was used for incubation, with water hardness adjusted upward to about 300 mg/l as $CaCO_3$ by seawater infusion (about 6% seawater). Incoming incubation water was split after seawater infusion, with one half of the supply "spiked" with the appropriate concentrations of the five heavy metals being tested. The "spiking" was accomplished by constantly metering concentrated metal solutions into the experimental incubation water stream at a carefully controlled rate. Each incubation water stream then flowed through a separate 5,000 gallon tank with an 8 hr retention time for chemical equilibration before entering the Heath incubators. Figure 2 shows the relationship of the elements making up the bioassay system.

Two exposure episodes were implemented for two weeks each to mimic expected conditions as closely as possible except that the experimental episodes occurred during the most sensitive portions of the incubation cycle, the first bracketing hatching and the second bracketing final yolk resorption. In this respect, the bioassay is again "worst case".

Water samples were taken periodically and sent to an independent chemical testing laboratory for analysis to monitor actual exposure levels of heavy metals and to obtain background information on the concentrations of these metals in Sheep Creek water and the sea water used to increase incubation water hardness. Samples were taken at a variety of points in the incubation water supply system as indicated in Figure 2.

The second exposure episode was terminated immediately prior to swim-out, and the experimental and control (now) fry were subjected to an extended salt water challenge as soon as possible following termination of metals exposure. Fry were removed from incubators and placed in small net pens in the 5,000 gal tanks for about 18 hrs to fill their swimbladders. They were then transported by truck on 15 March using standard hatchery procedures and placed in two square salt water pens measuring 7 ft on a side (343 ft^3) located at the DIPAC Thane pen site in Gasteneau Channel. Following salt water penning, fish were

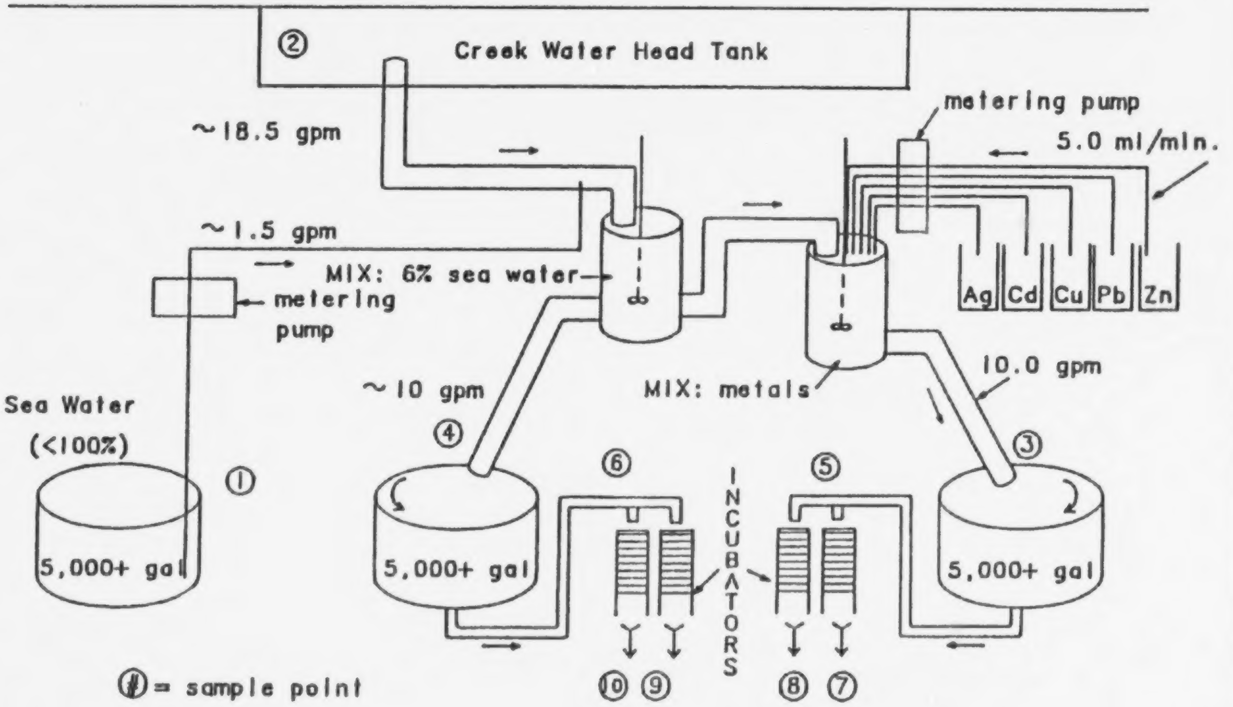

②
Creek Water Head Tank
~18.5 gpm
metering pump
5.0 ml/min.
~1.5 gpm
MIX: 6% sea water
metering
pump
Ag
Cd
Cu
Pb
Zn
~10 gpm
10.0 gpm
MIX: metals
Sea Water
(<100%)
①
④
③
⑥
⑤
INCUBATORS
5,000+ gal
5,000+ gal
5,000+ gal
⑩ ⑨
⑧ ⑦
#= sample point

FIGURE 2 GENERAL ORIENTATION OF THE ELEMENTS OF THE SHEEP CREEK BIOASSAY

8/23/90 DWG: BIOASSAY

placed on a standard feed regimen ("Bio-Diet"^{R}). Feeding was started at a rate of once per day and increased to three times daily as fish grew. Daily records were kept of mortalities and growth was monitored periodically. Experimental and control fish were held for observation in these pens for 64 days (until 18 May) and then released to the open ocean.

The effects of episodic exposure of the experimental group of developing eggs / alevins to the five metals were measured primarily in terms of survival through two phases of development: through the swim-out stage, which gives an indication of short term survival, and through the initial salt water rearing stage (until release to the open ocean), which constitutes a long term (over 60 days) salt water challenge and which gives a good indication of overall long term survival. Mortalities and developmental anomalies were carefully tallied for both groups. In addition, growth rates of the two groups of fish were monitored throughout the salt water challenge period.

Effects of episodic exposure to the five heavy metals were also measured by analyzing samples of alevins and (later) fry for whole-fish body burdens for each of the metals throughout the second episodic exposure and afterward throughout the 64-day salt water challenge period. Near the end of the salt water challenge period, body compartment metal burden analyses were also performed. Ten to fifteen individual fish were pooled for each sample. During the incubation phase, fish samples were drawn randomly from three of the six active trays in each stack, producing six samples each for the experimental and control groups. During the salt water challenge period, triplicate samples were randomly drawn from each of the two net pens, one containing experimental fish and one containing control fish.

RESULTS

Incubation Water Monitoring

Results of chemical analyses performed on incubation water from selected sampling points are presented in Table 1 for the first exposure episode and Table 2 for the second exposure episode. These results show that exposure levels for experimental eggs / alevins were maintained at or near the desired "target" concentrations throughout both exposure episodes. During the first exposure episode, unusually high levels of certain metals were detected in samples taken from water entering incubator stacks, especially for copper lead and zinc. Since some physical manipulation of the plumbing was necessary to obtain samples at these particular locations, and since no such unusually high concentrations of metals were detected in water leaving incubator stacks (where no manipulationof plumbing was necessary) it was

TABLE 1

SHEEP CREEK BIOASSAY -- FIRST EPISODIC EXPOSURE -- WATER ANALYSES

		SILVER (Ag)						CADMIUM (Cd)						COPPER (Cu)						LEAD (Pb)						ZINC (Zn)					
		STATION						STATION						STATION						STATION						STATION					
DATE	DAY	3	4	5	6	8	10	3	4	5	6	8	10	3	4	5	6	8	10	3	4	5	6	8	10	3	4	5	6	8	10
03 OCT	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	0	0	4	2	0	1	0	0	0	0	5	49	1	6	8	9	5
	0.04	3.6		0.0				2.3		0.3	0.0			21		0				24		1				55		16			
	0.25	4.5		2.5				2.4		1.4	0.0			23		1				17		11				29		25			
04	0.75	3.8		3.0				2.4		1.9	0.1			23		2				17		15				27		24			
	1.00	2.6	0.0	2.8	0.0	2.8	0.0	2.2	0.0	2.1	0.1	2.1	0.0	22	0	20	2	21	0	19	6	17	6	20	0	24	4	22	4	22	2
07	4.00	3.8	0.0	4.4	0.0	2.4	0.3	2.5	0.2	2.3	0.0	2.4	0.0	39	17	39	2	21	0	23	2	47	0	23	0	44	22	30	5	23	4
10	7.00	3.8	0.0	3.9	0.0	3.1	0.0	2.5	0.2	2.9	1.7	2.5	0.2	21	0	27	1	20	0	19	0	23	0	16	0	24	3	41	3	21	3
13	10.00	3.9	0.0	4.2	0.0	3.3	0.0	2.6	0.0	2.5	0.1	2.1	0.0	22	0	54	4	17	0	20	0	78	1	17	0	23	7	30	8	22	2
16	13.00	3.7	0.0	3.9	0.0	3.3	0.0	2.3	0.0	2.5	0.0	2.3	0.0	19	1	35	4	19	0	18	0	39	1	16	0	21	3	57	39	21	2
18	15.00	3.5	0.0	3.0	0.0	3.1	0.0	2.5	0.1	2.7	0.5	2.6	0.0	24	0	39	3	20	0	25	0	46	0	18	0	24	2	29	5	23	2
	15.04	0.3		4.1				0.2		2.7				0		55				0		68				2		35			
	15.25	0.0		1.0				0.0		1.4				1		25				0		9				2		16			
19	15.75	0.0		0.5				0.1		0.2				0		4				0		10				0		10			
	16.00	0.0	0.0	0.0		0.0	0.0	0.1	0.0	0.2		0.2	0.0	0	0	5		2	0	0	0	4		1	0	2	0	8		3	1

TABLE 2

SHEEP CREEK BIOASSAY -- SECOND EPISODIC EXPOSURE -- WATER ANALYSES

DATE	DAY	SILVER (Ag) STATION						CADMIUM (Cd) STATION					
		3	4	5	6	8	10	3	4	5	6	8	10
26 FEB	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
	0.04	3.9	0.0	0.5	0.0			2.4	0.1	0.4	0.0		
	0.25	4.0	0.0	2.1	0.0			2.3	0.0	1.4	0.0		
27	0.75	4.1	0.0	3.4	0.0			2.6	0.0	2.2	0.0		
	1.00	3.4	0.0	3.3	0.0	3.5	0.0	2.2	0.3	2.7	0.0	2.2	0.0
02 MAR	4.00	4.0	0.0	3.6	0.0	3.4	0.0	2.1	0.0	1.9	0.0	1.8	0.0
** 05	6.90	4.0	0.0	3.6	0.0	3.4	0.0	2.0	0.0	1.9	0.0	1.8	0.0
*** 05	7.00	0.0	0.0	3.0	0.0	2.7	0.0	0.0	0.0	1.7	0.0	1.6	0.0
06	8.00	3.3	0.0	2.9	0.0	3.0	0.0	2.1	0.0	1.8	0.0	1.9	0.0
08	10.00	4.3	0.0	3.8	0.0	3.6	0.0	1.9	0.0	1.8	0.0	1.7	0.0
11	13.00	4.1	0.0	4.1	0.0	4.1	0.0	2.0	0.1	2.5	0.1	2.1	0.0
13	15.00	4.1	0.0	3.6	0.0	0.0	0.0	2.1	0.0	2.1	0.0	2.1	0.0
	15.04	0.0	0.0	3.6	0.0			0.0	0.0	2.0	0.0		
	15.25	0.0	0.0	1.9	0.0			0.0	0.0	1.1	0.0		
14	15.75	0.0	0.0	0.3	0.0			0.0	0.0	0.1	0.0		
	16.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DATE	DAY	COPPER (Cu) STATION						LEAD (Pb) STATION						ZINC (Zn) STATION					
		3	4	5	6	8	10	3	4	5	6	8	10	3	4	5	6	8	10
26 FEB	0.00	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3	1	5	3
	0.04	21	0	4	0			20	0	3	0			22	0	4	0		
	0.25	21	0	12	0			20	0	11	0			23	0	14	1		
27	0.75	25	0	21	0			22	0	19	0			24	1	22	0		
	1.00	20	0	21	0	20	0	18	0	21	0	17	0	22	1	22	0	24	3
02 MAR	4.00	22	0	20	0	22	0	23	0	19	0	20	0	22	0	23	0	23	0
** 05	6.90	20	0	20	0	20	0	20	0	19	0	20	0	20	0	20	0	20	0
*** 05	7.00	0	0	17	0	17	0	0	0	15	0	16	1	0	0	18	1	17	1
06	8.00	20	0	17	0	19	1	21	0	17	0	17	0	22	0	18	1	20	0
08	10.00	23	0	19	0	20	0	19	0	19	0	18	0	23	1	21	1	21	0
11	13.00	20	0	22	0	21	1	18	0	18	0	18	0	18	0	20	1	21	0
13	15.00	23	0	23	0	24	0	20	0	19	0	22	0	22	0	21	0	23	1
	15.04	2	0	22	0			0	0	17	2			3	1	21	2		
	15.25	0	0	12	0			2	0	9	2			2	0	12	1		
14	15.75	0	0	3	0			1	2	2	6			2	0	3	2		
	16.00	0	0	2	0	3	0	0	1	2	3	2	0	1	0	2	1	4	2

** DUMMY VALUE ENTERED FOR PLOTTING PURPOSES: TARGET VALUE OR PREVIOUS VALUE, WHICHEVER IS LOWER

*** PUMP FAILURE (about 4 hours)

suspected that the analytical results from the water flowing into incubator stacks were sometimes anomalous. A change in the sampling procedure eliminating the need to manipulate plumbing appears to have also eliminated the unusually high detected metals concentrations for this sampling station, as can be seen in the analytical results for the second exposure episode.

An infusion pump failure occurred in the middle of the second exposure episode, lasting about 4 hr. This resulted in an interruption in the supply of metals into the incubation water supply, but because of the presence of the 5,000 gal tank between the point of metals infusion and the experimental incubators, only a slight, temporary decline in metals concentrations was detected at the experimental incubator inflow. The effect of the temporary pump failure on metals concentrations upstream and downstream of the 5,000 gal tank is clearly evident in the results of chemical analyses graphed in Figures 18 through 27.

Incubation Survival (Short Term)

Short term incubation mortalities and developmental anomalies tallied for each experimental and control incubator tray are presented in Tables 3 through 6, along with survival rates for each tray. Mortalities are presented according to the life stage at which death occurred and developmental anomalies have been assigned to certain categories ("circ." = deformity into the shape of a circle; "bloat" = distended coelomic cavity; "pop eye" = protruding eyes and shortened snout; "kink" = spinal deformity; "twin" = siamese twin; "spiral" = severe spinal deformity resulting in a corkscrew appearance). Overall survival through the incubation phase was very high for both the experimental and the control groups, exceeding survival rates expected for most hatcheries under any conditions, and exceeding the survival rates generally experienced at the Sheep Creek Hatchery. If all anomalies and mortalities over the incubation cycle (short term results) are summed, a slightly lower survival rate is obtained for the experimental group: 98.7% survival through swim-out for the experimental group and 99.1% survival through swim-out for the control group. A two-way ANOVA comparing life stage at mortality and heavy metals exposure indicates that there was no significant difference in life stage at mortality between the two groups ($P > 0.16$) but that the albeit small difference in overall survival through swim-out between the two groups was significant ($P < 0.02$). The short term survival rate of the experimental group is well within the most conservative of the two criteria for continuing successful operation of the Sheep Creek Hatchery set before initiation of the bioassay (at least 94.17% survival of the experimental group, given the 99.13% survival of the control group).

TABLE 3

SHEEP CREEK BIOASSAY
RECORD OF INCUBATION MORTALITIES (PARTIAL)

BOTH EPISODIC EXPOSURES THROUGH PONDING—03 OCTOBER 1989 THROUGH 14 MARCH 1990

EXPERIMENTAL GROUP

STACK "A" — START: 42,966 eggs. Mortality thru eyed stage: 477 (1.1%)

		MORTALITIES										SURVIVALS	
Tray	Load	eggs	sac fry	adv. fry	mat. fry	circ.	bloat	pop eye	kink	other	TOTAL (%)	TOTAL	(%)
2	7,000	41	2	3	5	-	-	1	4	2 twin 2 spiral	60 (0.86)	6,940	(99.14)
3	7,000	16	6	7	5	-	1	9	2	1 twin 3 spiral	50 (0.71)	6,950	(99.29)
4	7,000	7	17	17	10	-	-	8	1	-	60 (0.86)	6,940	(99.14)
5	7,000	20	64	70	12	3	-	17	4	4 twin 1 coag. yolk	195 (2.79)	6,805	(97.21)
6	7,000	49	17	28	13	3	-	8	4	1 2-head	123 (1.76)	6,877	(98.24)
7	7,489	24	7	2	7	-	-	3	1	2 2-head	46 (0.61)	6,954	(99.34)
TOTAL (%)	42,489	157 (0.37)	113 (0.27)	127 (0.30)	52 (0.12)	6 (0.01)	1 (0.00)	46 (0.11)	16 (0.04)	16 (0.04)	534 (1.26)	41,955	(98.74)

Total cumulative mortality, fertilization through ponding = 1,011/42,966 = 2.35%

TABLE 4

SHEEP CREEK BIOASSAY
RECORD OF INCUBATION MORTALITIES (PARTIAL)

BOTH EPISODIC EXPOSURES THROUGH PONDING—03 OCTOBER 1989 THROUGH 14 MARCH 1990

EXPERIMENTAL GROUP

STACK "B" — START: 39,612 eggs. Mortality thru eyed stage: 462 (1.2%)

		MORTALITIES										SURVIVALS	
Tray	Load	eggs	sac fry	adv. fry	mat. fry	circ.	bloat	pop eye	kink	other	TOTAL (%)	TOTAL	(%)
2	7,000	31	1	2	15	-	-	1	1	-	51 (0.73)	6,949	(99.27)
3	7,000	57	9	7	4	-	-	3	1	2 spiral 1 twin	84 (1.20)	6,916	(98.80)
4	7,000	81	13	11	8	-	-	26	1	3 spiral	143 (2.04)	6,857	(97.96)
5	7,000	12	14	68	11	1	1	37	1	1 twin 3 spiral	149 (2.13)	6,851	(97.87)
6	7,000	12	26	11	7	-	1	3	5	2 twin 5 spiral	72 (1.03)	6,928	(98.97)
7	4,150	16	7	1	5	-	-	-	1	2 twin 1 spiral	33 (0.80)	4,117	(99.20)
TOTAL (%)	39,150	209 (0.53)	70 (0.18)	100 (0.26)	50 (0.13)	1 (0.00)	2 (0.01)	70 (0.18)	10 (0.03)	20 (0.05)	532 (1.36)	38,618	(98.64)

Total cumulative mortality, fertilization through ponding = 994/39,612 = 2.51%

TABLE 5

SHEEP CREEK BIOASSAY
RECORD OF INCUBATION MORTALITIES (PARTIAL)

BOTH EPISODIC EXPOSURES THROUGH PONDING—03 OCTOBER 1989 THROUGH 14 MARCH 1990

CONTROL GROUP

STACK "C" — START: 41,548 eggs. Mortality thru eyed stage: 368 (0.9%)

		MORTALITIES										SURVIVALS	
Tray	Load	eggs	sac fry	adv. fry	mat. fry	circ.	bloat	pop eye	kink	other	TOTAL (%)	TOTAL	(%)
2	7,000	15	1	7	-	-	1	1	-	1 2-head	26 (0.37)	6,974	(99.63)
3	7,000	12	5	6	10	-	2	-	1	1 spiral 1 twin	38 (0.54)	6,962	(99.46)
4	7,000	11	2	5	18	-	-	1	3	3 spiral	43 (0.61)	6,957	(99.39)
5	7,000	11	36	27	11	-	-	8	2	1 twin 2 spiral	98 (1.40)	6,902	(98.60)
6	7,000	24	19	20	8	-	-	6	-	1 twin 1 spiral	79 (1.13)	6,921	(98.87)
7	6,180	33	5	4	8	-	-	-	-	2 spiral	52 (0.84)	6,128	(99.16)
TOTAL (%)	41,180	106 (0.26)	68 (0.17)	69 (0.17)	55 (0.13)	- (0.00)	3 (0.01)	16 (0.04)	6 (0.01)	13 (0.03)	336 (0.82)	40,844	(99.18)

Total cumulative mortality, fertilization through ponding = 704/41,548 = 1.69%

TABLE 6

SHEEP CREEK BIOASSAY
RECORD OF INCUBATION MORTALITIES (PARTIAL)

BOTH EPISODIC EXPOSURES THROUGH PONDING—03 OCTOBER 1989 THROUGH 14 MARCH 1990

CONTROL GROUP

STACK "D" — START: 38,251 eggs. Mortality thru eyed stage: 370 (1.0%)

		MORTALITIES										SURVIVALS	
Tray	Load	eggs	sac fry	adv. fry	mat. fry	circ.	bloat	pop eye	kink	other	TOTAL (%)	TOTAL	(%)
2	7,000	11	3	2	12	-	-	1	1	1 spiral	32 (0.46)	6,968	(99.54)
3	7,000	23	6	4	12	-	-	3	2	3 spiral 2 twin 1 2-head	56 (0.80)	6,944	(99.20)
4	7,000	8	13	11	14	-	2	3	-	1 spiral	52 (0.74)	6,948	(99.26)
5	7,000	15	41	31	15	-	-	19	1	1 2-head	123 (1.76)	6,877	(98.24)
6	7,000	20	11	5	5	-	-	5	1	1 spiral 1 2-head	49 (0.70)	6,951	(99.30)
7	2,881	19	4	3	6	-	-	1	-	3 spiral	36 (1.25)	2,845	(98.75)
TOTAL (%)	37,881	96 (0.25)	78 (0.21)	56 (0.14)	64 (0.17)	- (0.00)	2 (0.01)	32 (0.08)	5 (0.01)	15 (0.04)	348 (0.92)	37,533	(99.08)

Total cumulative mortality, fertilization through ponding = 768/38,251 = 2.01%

Salt Water Challenge Survival and Growth (Long Term)

Results of daily monitoring of mortalities of experimental and control fish are displayed graphically in Figure 3. A certain "drop-out" mortality rate in excess of incidental on-going mortality is normal and expected for chum fry after salt water penning. After about 20 days of salt water holding, control fish began to die at a rate which was higher than normal, while experimental fish maintained a lower than normal mortality rate. For comparative purposes, Figure 3 includes data for a large group (3 million) of chum fry from the Gasteneau Hatchery being held at the Thane pen site. Although the large group was released after 30 days, the drop-out rate for these fish during that time was between that for the experimental group and that for the control group from the bioassay.

Results of periodic monitoring of growth rates of experimental and control fish are displayed graphically in Figure 4. The experimental group out-performed the control group in all respects, feeding more vigorously and aggressively, growing faster, surviving at a significantly higher rate, and, in general, appearing more robust and healthy. This difference was considerably more obvious than the slightly lower short term survival rate observed for the experimental group during the incubation phase of this bioassay.

Whole Fish and Body Copmpartment Metal Burdens

Results of whole body burden tissue analyses are presented in Table 7 and displayed graphically in Figures 5 through 9. Body compartment analyses for the five heavy metals used in the Sheep Creek bioassay were conducted for the last two fish samples taken (07 May and 17 May; 53 and 63 days after salt water penning). Results of these analyses of body compartments, muscle, bone (operculum plus spine) and whole viscera are presented in Table 8 and are depicted graphically in Figures 10 through 14.

Fish Food Analysis

The initial declines in experimental group whole body burdens of cadmium, copper and zinc following salt water penning were followed by steady increases in tissue concentrations of these metals in both experimental and control fish after about 20 days. This observation led to the suspicion of an exogenous source of these metals. Ongoing analyses ruled out sea water as the source. The only other obvious candidate was the food supply.

A random sample of the standard regimen (BioDiet^{R}, BioProducts, Inc.) used to feed both experimental and control fish during the 64-day salt water challenge period was analyzed for all five

FIGURE 3

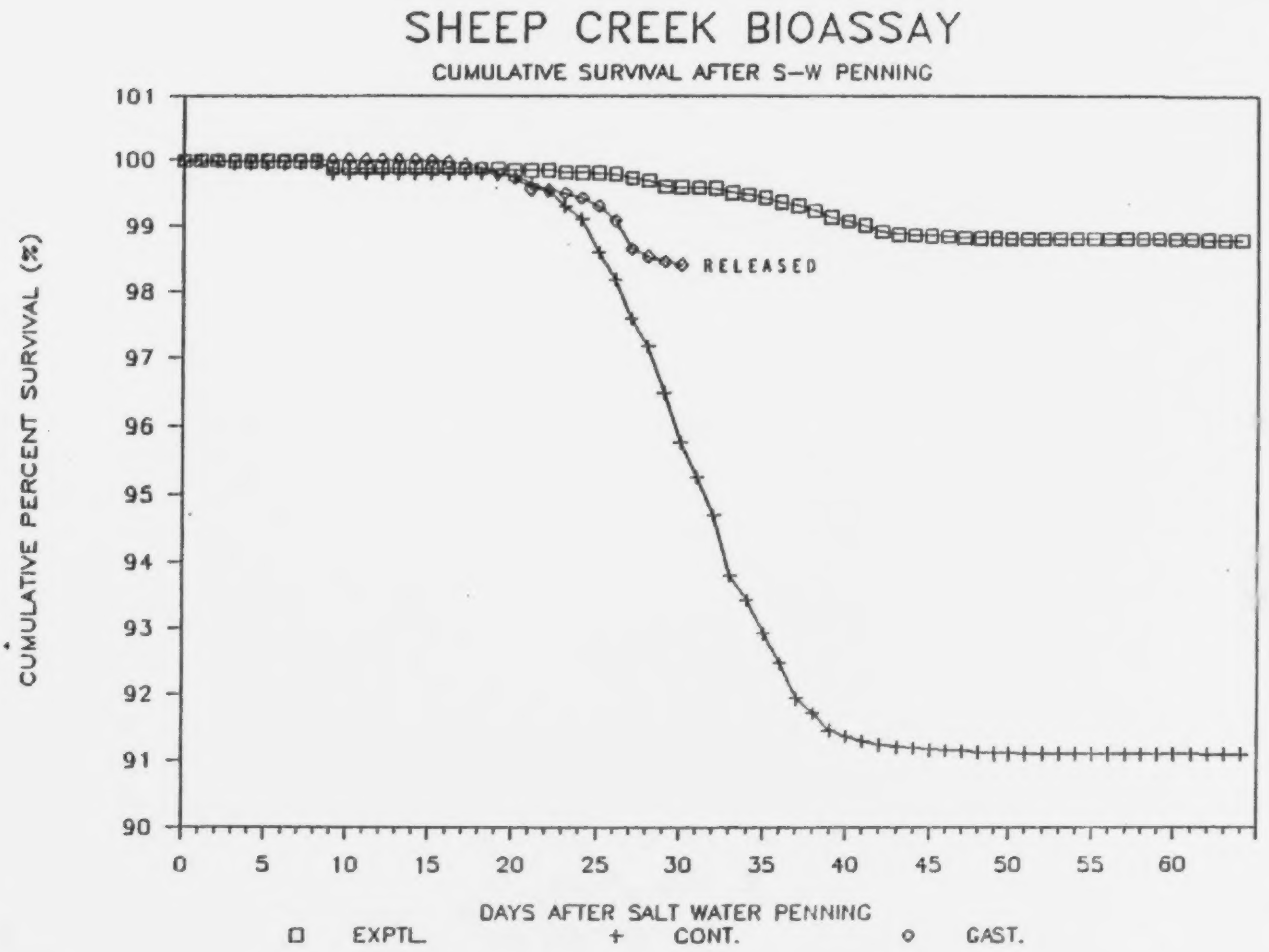

SHEEP CREEK BIOASSAY
CUMULATIVE SURVIVAL AFTER S-W PENNING
CUMULATIVE PERCENT SURVIVAL (%)
101
100
99
98
97
96
95
94
93
92
91
90
0
5
10
15
20
25
30
35
40
45
50
55
60
RELEASED
DAYS AFTER SALT WATER PENNING
□ EXPTL.
+ CONT.
◇ GAST.

FIGURE 4

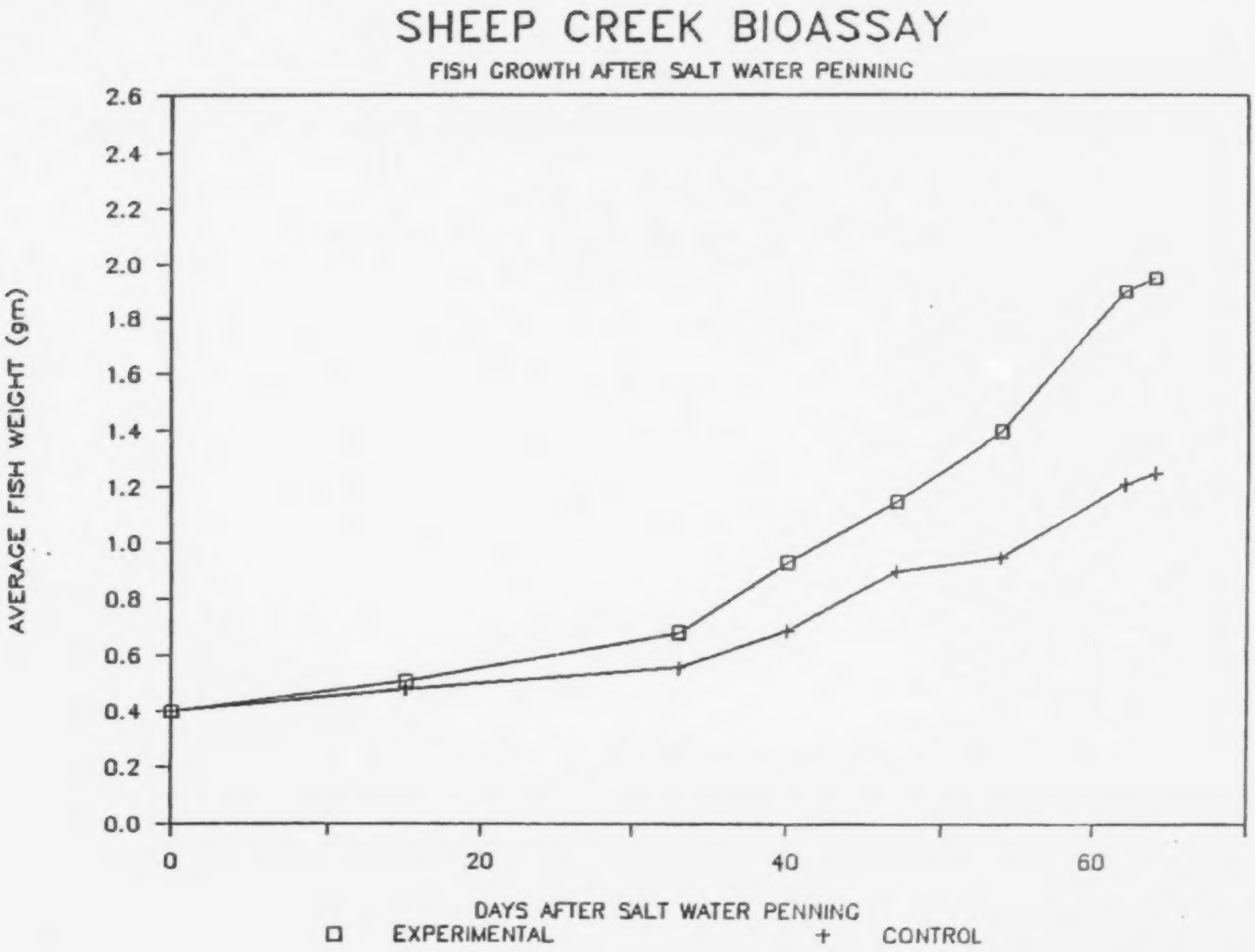

SHEEP CREEK BIOASSAY
FISH GROWTH AFTER SALT WATER PENNING
AVERAGE FISH WEIGHT (gm)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
DAYS AFTER SALT WATER PENNING
□ EXPERIMENTAL
+ CONTROL

TABLE 5

SHEEP CREEK BIOASSAY — WHOLE FISH METALS CONCENTRATIONS

	SILVER (Ag)				CADMIUM (Cd)				COPPER (Cu)				LEAD (Pb)				ZINC (Zn)			
	EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL	
	12		6		4		3		370		890		21		10		10,800		33,400	
	21	MEAN =	3	MEAN =	14	MEAN =	1	MEAN =	1120	MEAN =	910	MEAN =	43	MEAN =	10	MEAN =	23,400	MEAN =	35,400	MEAN =
DAY 0	23	21.833	3	3.667	4	5.333	3	3.667	760	746.7	1040	856.7	21	24.7	24	13.3	14,700	17,833	27,400	37,167
	32		5		2		7		630		790		22		10		11,300		18,400	
	23	SD =	3	SD =	3	SD =	5	SD =	710	SD =	950	SD =	18	SD =	16	SD =	23,300	SD =	61,300	SD =
	20	6.432	2	1.506	5	4.367	1	2.160	890	196.6	830	155.5	23	9.1	10	5.8	22,700	6,100	41,200	11,450
MTLS ON—>																				
	20		2		4		1		890		640		40		10		10,900		8,220	
	22	MEAN =	2	MEAN =	4	MEAN =	2	MEAN =	890	MEAN =	620	MEAN =	38	MEAN =	10	MEAN =	8,400	MEAN =	17,000	MEAN =
DAY 2	26	26.500	2	2.333	5	4.500	1	1.333	690	883.3	590	625.0	41	41.0	10	10.0	13,200	11,747	7,300	10,410
	32		3		5		1		970		550		45		10		11,500		8,350	
	31	SD =	3	SD =	4	SD =	2	SD =	910	SD =	620	SD =	47	SD =	10	SD =	12,500	SD =	10,000	SD =
	25	3.391	2	0.516	5	0.548	1	0.516	950	100.1	730	60.2	35	5.3	10	0.0	13,300	1,917	9,600	3,610
	61		6		10		4		1270		810		200		60		13,000		10,300	
	50	MEAN =	6	MEAN =	9	MEAN =	3	MEAN =	1130	MEAN =	835	MEAN =	140	MEAN =	60	MEAN =	13,200	MEAN =	11,300	MEAN =
DAY 7	58	54.333	3	4.2	8	7.833	4	3.500	994	1011.3	852	820.7	110	156.7	50	46.7	11,000	11,100	10,300	10,932
	53		4		8		3		1000		853		120		50		11,400		12,800	
	44	SD =	** 206	SD =	5	SD =	4	SD =	781	SD =	814	SD =	150	SD =	30	SD =	9,040	SD =	10,800	SD =
	50	7.448	4	1.095	7	1.722	3	0.548	883	165.1	756	28.7	220	44.1	30	13.7	8,040	2,100	9,490	1,107
	67		6		7		3		960		870		300		60		10,000		10,500	
	61	MEAN =	6	MEAN =	7	MEAN =	2	MEAN =	890	MEAN =	700	MEAN =	270	MEAN =	50	MEAN =	9,190	MEAN =	12,700	MEAN =
DAY 15	59	63.333	5	3.667	8	9.833	4	3.167	920	992.5	810	846.7	250	261.7	60	36.7	10,500	11,115	15,300	12,167
	60		4		11		4		940		790		290		60		12,000		10,900	
	73	SD =	2	SD =	10	SD =	1	SD =	1150	SD =	910	SD =	300	SD =	10	SD =	11,000	SD =	11,400	SD =
	60	5.279	3	1.033	10	3.130	5	1.472	1040	98.1	790	221.0	250	58.7	40	13.7	12,000	1,201	12,200	4,452
MTLS OFF—>																				
	83	MEAN =	5	MEAN =	** 1120	MEAN =	2	MEAN =	1340	MEAN =	700	MEAN =	300	MEAN =	20	MEAN =	11,000	MEAN =	10,400	MEAN =
DAY 17	56	63.333	4	4.000	9	13.500	3	2.333	880	1166.7	850	796.7	270	346.7	10	13.3	8,130	10,010	9,320	9,703
	73	SD =	3	SD =	18	SD =	2	SD =	1280	SD =	840	SD =	470	SD =	10	SD =	11,900	SD =	9,400	SD =
		9.504		1.000		6.364		0.577		250.1		83.9		107.9		5.8		1,900		516
TO SW PENS—>																				
	40	MEAN =	6	MEAN =	7	MEAN =	3	MEAN =	750	MEAN =	650	MEAN =	160	MEAN =	20	MEAN =	7,790	MEAN =	9,600	MEAN =
DAY 18	53	54.333	6	6.000	9	9.667	10	5.000	880	943.3	1050	810.0	170	170.0	60	33.3	7,470	8,013	9,600	9,000
	70	SD =	6	SD =	13	SD =	2	SD =	1200	SD =	730	SD =	180	SD =	20	SD =	8,780	SD =	7,840	SD =
		15.044		0.000		3.055		4.359		231.6		211.7		10.0		23.1		683		1,247
	31	MEAN =	3	MEAN =	10	MEAN =	2	MEAN =	423	MEAN =	556	MEAN =	170	MEAN =	40	MEAN =	8,450	MEAN =	8,050	MEAN =
DAY 22	21	26.000	2	2.333	6	7.667	2	1.667	464	812.3	552	502.7	300	196.7	24	25.3	8,840	8,667	8,310	8,327
	26	SD =	2	SD =	7	SD =	1	SD =	550	SD =	398	SD =	120	SD =	12	SD =	8,710	SD =	8,220	SD =
		5.000		0.577		2.082		0.577		107.6		90.7		92.9		14.0		1,335		1,427
	18	MEAN =	2	MEAN =	7	MEAN =	6	MEAN =	518	MEAN =	552	MEAN =	73	MEAN =	30	MEAN =	6,770	MEAN =	6,990	MEAN =
DAY 36	25	20.667	4	3.000	10	8.667	5	6.000	692	596.0	555	584.0	116	97.7	27	34.0	7,500	7,367	6,910	7,733
	19	SD =	3	SD =	9	SD =	7	SD =	578	SD =	667	SD =	110	SD =	45	SD =	7,830	SD =	9,290	SD =
		3.786		1.000		1.528		1.000		88.4		77.1		21.6		9.6		542		1,435
	22	MEAN =	3	MEAN =	19	MEAN =	11	MEAN =	1400	MEAN =	970	MEAN =	66	MEAN =	38	MEAN =	13,600	MEAN =	17,500	MEAN =
DAY 57	19	20.333	4	3.333	16	18.333	11	11.333	940	1146.7	1120	1046.7	87	85.0	58	48.7	12,700	14,600	14,600	15,267
	20	SD =	3	SD =	20	SD =	12	SD =	1100	SD =	1050	SD =	102	SD =	50	SD =	17,500	SD =	13,700	SD =
		1.528		0.577		2.517		0.577		233.5		75.1		18.1		10.1		2,551		1,985
	9	MEAN =	5	MEAN =	13	MEAN =	16	MEAN =	650	MEAN =	1040	MEAN =	140	MEAN =	60	MEAN =	19,900	MEAN =	18,700	MEAN =
DAY 71	7	10.333	0	3.333	13	15.000	16	17.000	690	810.0	1160	1220.7	70	100.0	60	56.7	22,600	20,167	18,600	20,067
	15	SD =	5	SD =	19	SD =	19	SD =	1090	SD =	1460	SD =	90	SD =	50	SD =	18,000	SD =	22,900	SD =
		4.163		2.887		3.464		1.732		158.5		227.4		36.1		5.8		2,312		2,456
	14	MEAN =	4	MEAN =	37	MEAN =	15	MEAN =	1220	MEAN =	867	MEAN =	279	MEAN =	110	MEAN =	17,500	MEAN =	33,700	MEAN =
DAY 81	12	12.667	2	3.000	12	21.667	20	17.333	749	957.7	971	929.3	75	148.0	120	106.7	14,400	19,933	20,000	23,633
	12	SD =	** 114	SD =	16	SD =	17	SD =	904	SD =	950	SD =	90	SD =	90	SD =	27,900	SD =	17,200	SD =
		1.155		1.414		13.429		2.517		240.0		55.0		113.6		15.3		7,071		8,830

** OBVIOUS CONTAMINATION OR ABERRANT VALUE...NOT USED TO COMPUTE MEANS OR STANDARD DEVIATIONS

FIGURE 5

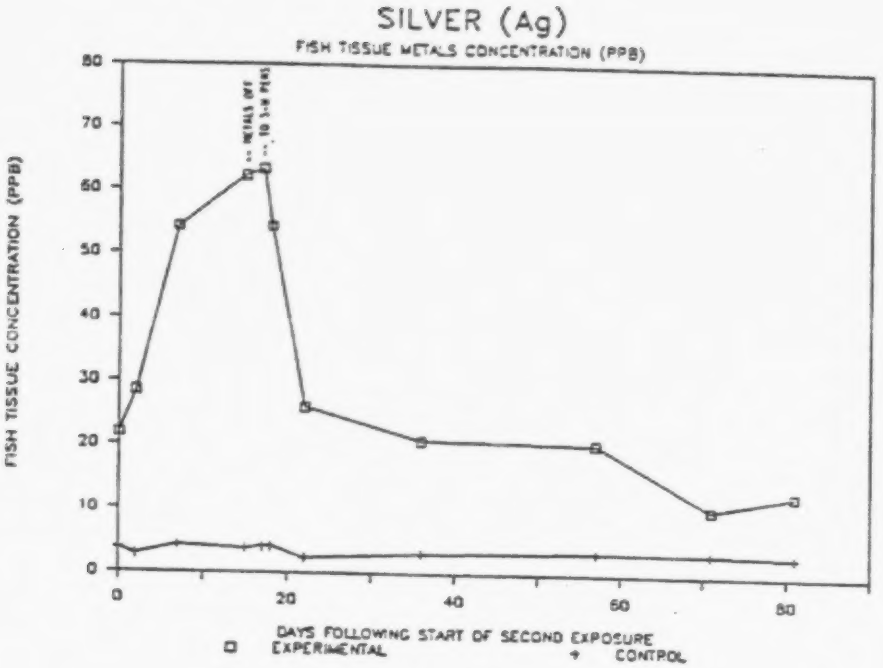


FIGURE 6

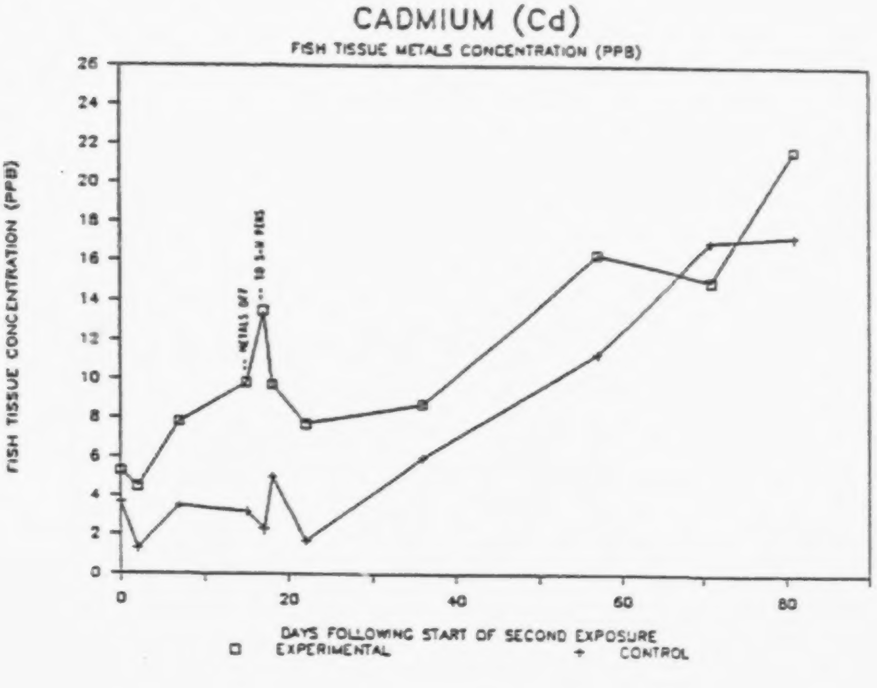


FIGURE 7

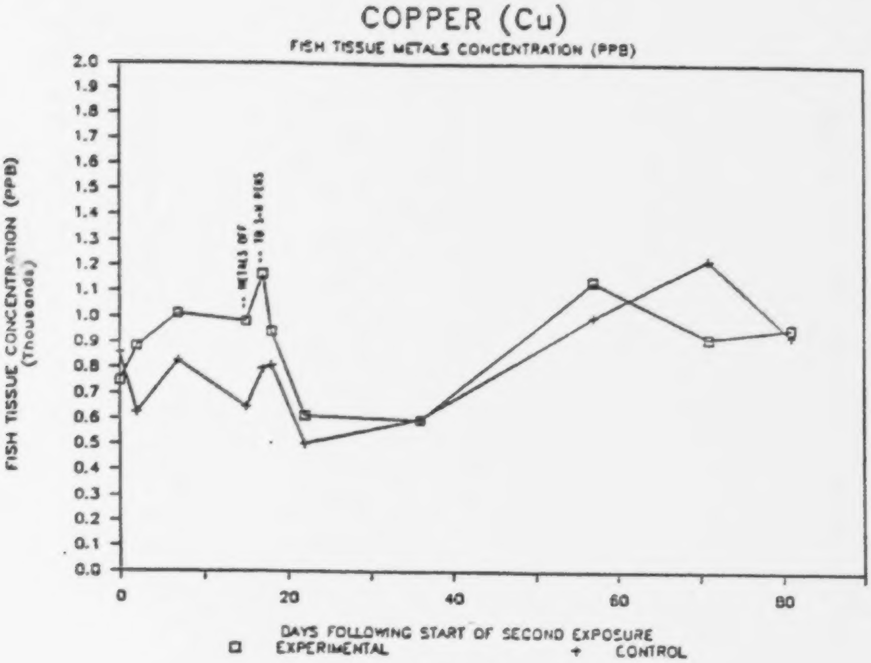


FIGURE 8

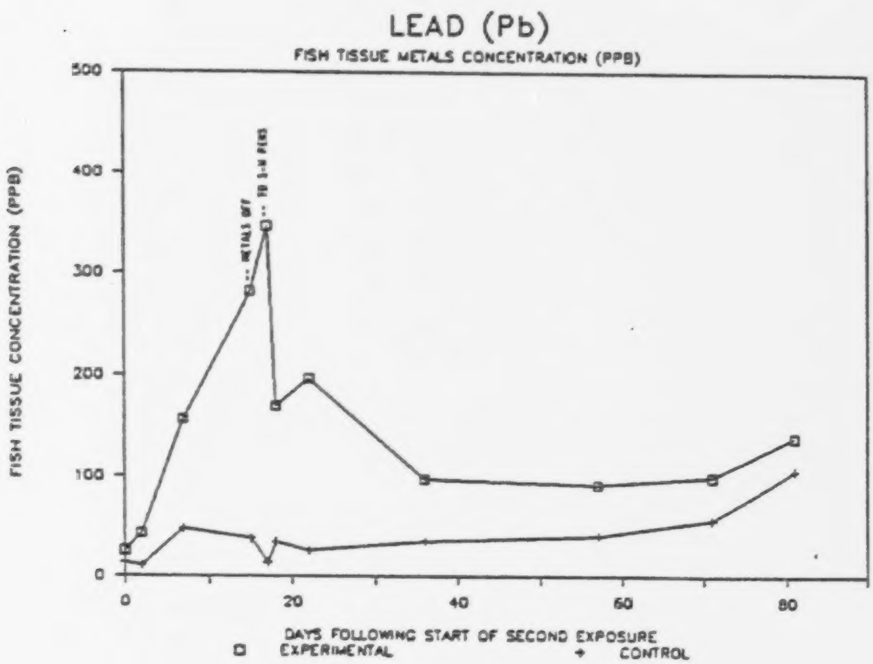


FIGURE 9

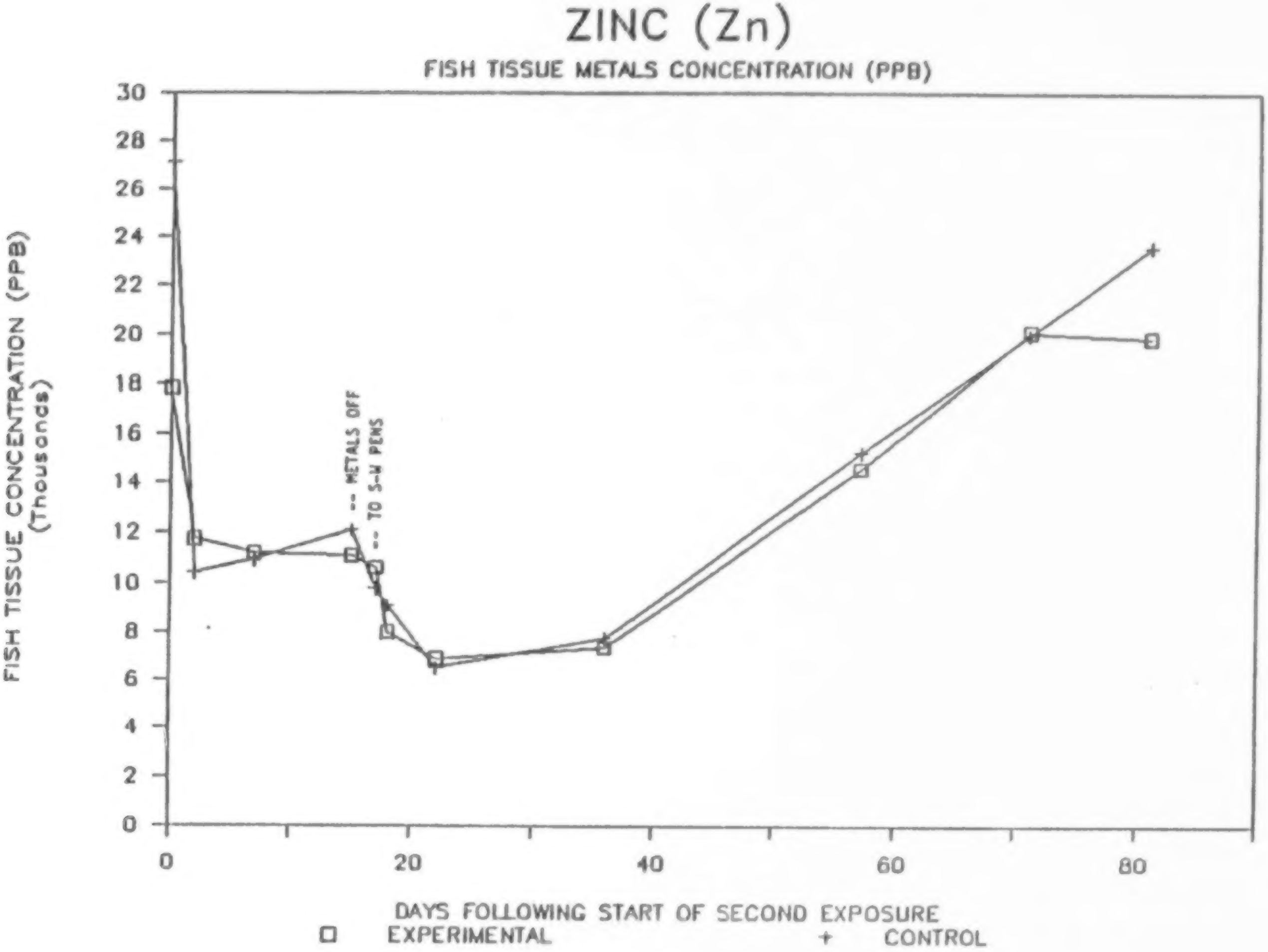

ZINC (Zn)
FISH TISSUE METALS CONCENTRATION (PPB)
FISH TISSUE CONCENTRATION (PPB)
(Thousands)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
-- METALS OFF
-- TO S-W PENS
0
20
40
60
80
DAYS FOLLOWING START OF SECOND EXPOSURE
□ EXPERIMENTAL
+ CONTROL

TABLE 6

SHEEP CREEK BIOASSAY -- BODY COMPARTMENT METALS CONCENTRATIONS

	SILVER (Ag)				CADMIUM (Cd)				COPPER (Cu)				LEAD (Pb)				ZINC (Zn)			
	EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL		EXPERIMENTAL		CONTROL	
WHOLE BODY																				
	9	MEAN :	5	MEAN :	13	MEAN :	16	MEAN :	856	MEAN :	1040	MEAN :	140	MEAN :	60	MEAN :	19,900	MEAN :	18,700	MEAN :
DAY 71	7	10.333	0	3.333	13	15.000	16	17.000	809	918.3	1160	1226.7	70	100.0	60	56.7	22,600	20,167	18,600	20,067
	15	SD :	5	SD :	19	SD :	19	SD :	1090	SD :	1480	SD :	90	SD :	50	SD :	18,000	SD :	22,900	SD :
		4.163		2.887		3.464		1.732		150.5		227.4		36.1		5.8		2,312		2,454
	14	MEAN :	4	MEAN :	37	MEAN :	15	MEAN :	1220	MEAN :	867	MEAN :	270	MEAN :	110	MEAN :	17,500	MEAN :	33,700	MEAN :
DAY 81	12	12.667	2	3.000	12	21.667	20	17.333	749	957.7	971	929.3	60	140.0	120	106.7	14,400	19,933	20,000	23,633
	12	SD :	** 114	SD :	16	SD :	17	SD :	904	SD :	950	SD :	90	SD :	90	SD :	27,900	SD :	17,200	SD :
		1.155		1.414		13.429		2.517		240.0		55.0		113.6		15.3		7,071		8,830
MUSCLE																				
	0	MEAN :	2	MEAN :	4	MEAN :	8	MEAN :	436	MEAN :	661	MEAN :	20	MEAN :	40	MEAN :	10,700	MEAN :	11,400	MEAN :
DAY 71	2	1.667	0	1.333	7	5.333	8	7.333	809	551.3	625	626.3	20	20.0	30	30.0	12,100	10,933	13,000	12,000
	3	SD :	2	SD :	5	SD :	6	SD :	409	SD :	593	SD :	20	SD :	20	SD :	10,000	SD :	11,600	SD :
		1.528		1.155		1.528		1.155		223.6		34.0		0.0		10.0		1,069		872
	2	MEAN :	2	MEAN :	8	MEAN :	8	MEAN :	520	MEAN :	1370	MEAN :	40	MEAN :	30	MEAN :	9,780	MEAN :	13,100	MEAN :
DAY 81	7	3.667	6	3.667	6	7.333	20	14.000	549	509.7	633	783.7	30	33.3	30	60.0	11,200	10,200	9,780	11,093
	2	SD :	3	SD :	8	SD :	14	SD :	460	SD :	348	SD :	30	SD :	120	SD :	9,620	SD :	10,400	SD :
		2.887		2.082		1.155		6.000		45.4		527.4		5.8		52.0		870		1,765
BONE																				
	2	MEAN :	0	MEAN :	10	MEAN :	8	MEAN :	466	MEAN :	552	MEAN :	100	MEAN :	50	MEAN :	18,400	MEAN :	23,100	MEAN :
DAY 71	3	3.000	0	0.667	12	9.667	6	7.333	558	488.7	589	591.0	100	103.3	30	33.3	22,100	19,767	18,400	21,167
	4	SD :	2	SD :	7	SD :	8	SD :	442	SD :	632	SD :	110	SD :	20	SD :	18,800	SD :	22,000	SD :
		1.000		1.155		2.517		1.155		61.2		40.0		5.8		15.3		2,031		2,458
	4	MEAN :	3	MEAN :	22	MEAN :	10	MEAN :	396	MEAN :	480	MEAN :	160	MEAN :	90	MEAN :	19,500	MEAN :	19,100	MEAN :
DAY 81	3	3.333	** 362	3.000	7	12.333	14	9.667	1210	725.7	605	488.7	110	133.3	100	73.3	26,600	22,000	23,100	20,333
	3	SD :	** 329	SD :	8	SD :	5	SD :	571	SD :	381	SD :	130	SD :	30	SD :	19,900	SD :	18,800	SD :
		0.577		N/A		8.386		4.509		428.5		112.3		25.2		37.9		3,989		2,401
WHOLE VISCERA																				
	60	MEAN :	6	MEAN :	40	MEAN :	49	MEAN :	3630	MEAN :	3310	MEAN :	230	MEAN :	280	MEAN :	24,100	MEAN :	27,000	MEAN :
DAY 71	38	50.333	5	5.667	43	41.667	42	43.000	2500	3143.3	2830	2906.7	220	263.3	240	233.3	25,500	25,133	23,300	24,567
	53	SD :	6	SD :	42	SD :	38	SD :	3300	SD :	2580	SD :	340	SD :	180	SD :	25,800	SD :	23,400	SD :
		11.240		0.577		1.528		5.568		581.1		371.0		66.6		50.3		907		2,108
	65	MEAN :	4	MEAN :	38	MEAN :	43	MEAN :	2810	MEAN :	2790	MEAN :	280	MEAN :	230	MEAN :	35,500	MEAN :	21,900	MEAN :
DAY 81	53	58.000	10	9.333	34	37.667	29	42.000	2850	2743.3	1670	2576.7	240	246.7	220	233.3	21,200	26,167	19,200	22,267
	56	SD :	14	SD :	41	SD :	54	SD :	2570	SD :	3270	SD :	220	SD :	250	SD :	21,800	SD :	25,700	SD :
		6.245		5.033		3.512		12.530		151.4		821.1		30.6		15.3		8,088		3,265

** OBVIOUS CONTAMINATION OR ABERRANT VALUE...NOT USED TO COMPUTE MEANS OR STANDARD DEVIATIONS

FIGURE 10

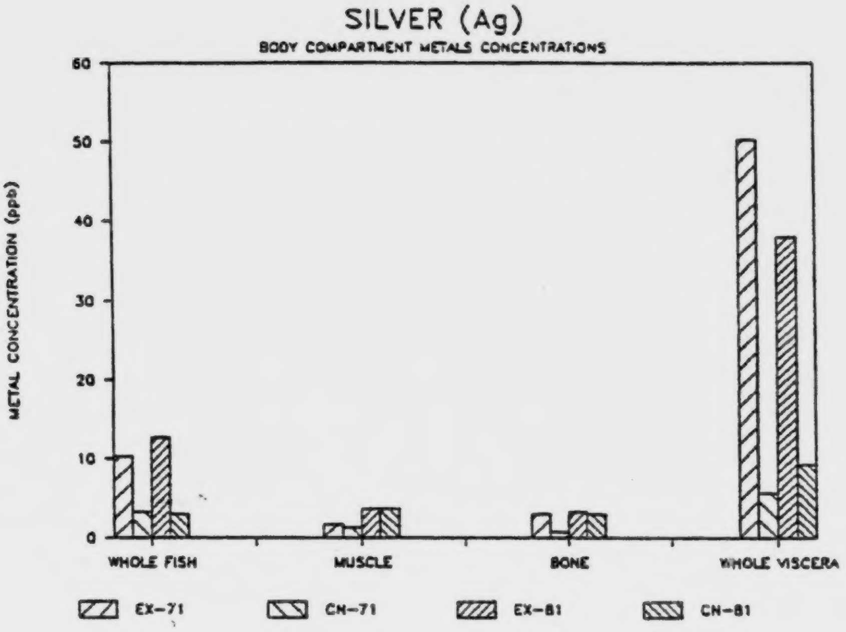


FIGURE 11

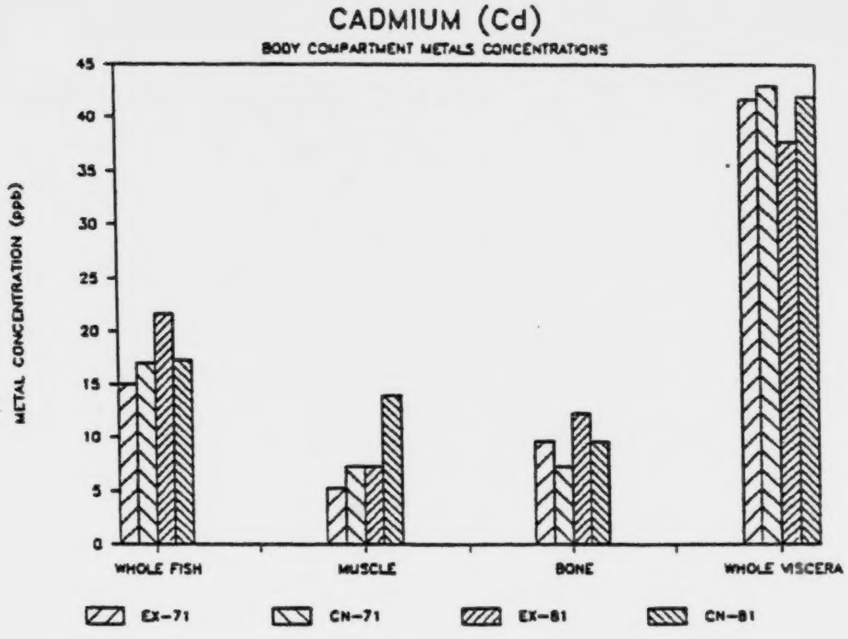


FIGURE 12

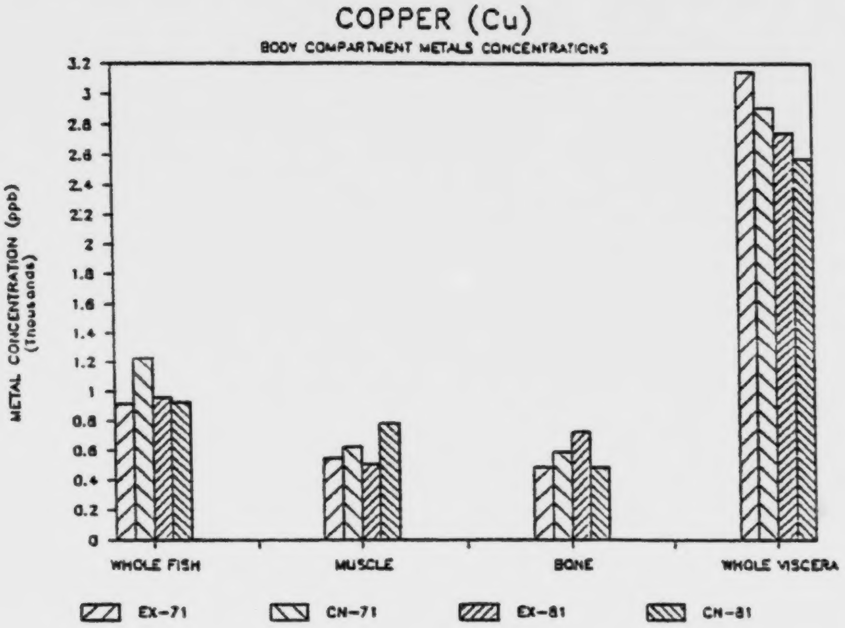


FIGURE 13

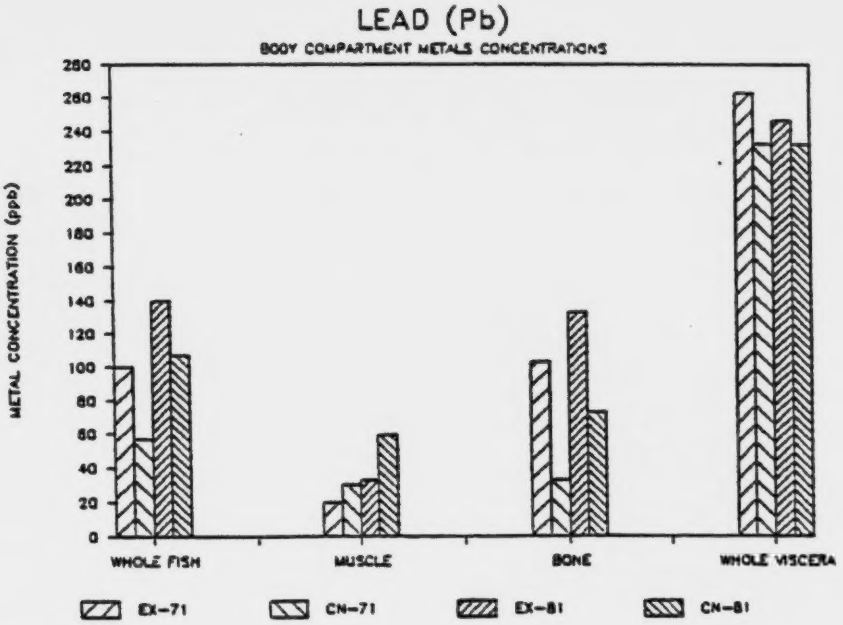
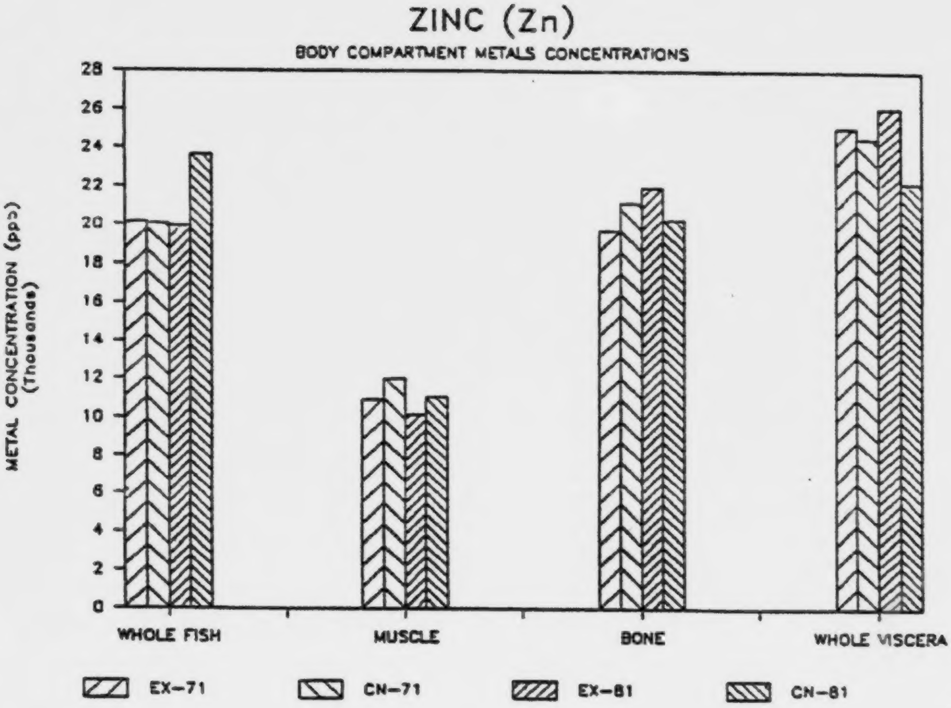


FIGURE 14


ZINC (Zn)
BODY COMPARTMENT METALS CONCENTRATIONS
METAL CONCENTRATION (ppm)
(Thousands)
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
WHOLE FISH
MUSCLE
BONE
WHOLE VISCERA
EX-71
CN-71
EX-81
CN-81

heavy metals used in the bioassay. The results of this analysis are given below, along with the target concentrations for experimental group incubation water (expected A-J mine tailings pond water concentrations) for comparative purposes:

METAL	FISH FOOD CONCENTRATION (ppb)	INCUBATION WATER CONCENTRATION (ppb)
Silver	2	4
Cadmium	255	2
Copper	6,670	20
Lead	1,320	20
Zinc	96,000	20

Each of these metals except silver is present in the food supply at concentrations far greater than the target concentrations used to simulate A-J mine tailings pond water in the bioassay. Likewise, each of these metals except silver exhibits a steady rise in tissue concentrations during the salt water challenge period in both experimental and control fish. These results indicate that the commercially prepared food supply was indeed the source of enhanced tissue levels of cadmium, copper and zinc, and later lead, observed in both groups after salt water penning.

It was discovered through conversations with the fish food manufacturer that a dietary copper supplement of about 1,500 ppb, in addition to copper already present in the formula, is added to the standard regimen. Copper is added because it is an important co-enzyme, notably in the formation of elastin. About 75,000 ppb (75 ppm) zinc is also added as a dietary supplement, bringing the total concentration in the food to nearly 100,000 ppb (100 ppm). Zinc is added to the standard regimen because it is an important co-enzyme for a variety of metabolic functions.

DISCUSSION

Results of this bioassay demonstrate that exposure of developing chum salmon eggs / alevins to concentrations of the five heavy metals of concern at or above concentrations expected for A-J mine tailings pond water, when incubation water hardness is adjusted to about 300 mg/l as $CaCO_3$, does not impair development or survival to the extent that hatchery operations would be jeopardized. On the contrary, experimental fry exhibited a very high survival rate by any standard and appeared very robust and healthy throughout incubation and an extended (64-day) salt water challenge period.

The small but significant difference in short term survival rates of the experimental and control groups suggests that exposure to heavy metals may have influenced development of experimental eggs and alevins. The patterns of mortalities observed suggest that if such an effect occurred, it was expressed at the advanced fry stage, when many of the observed "pop-eye" mortalities occurred. However, other uncontrollable variables such as flow patterns in incubators, differences in the age and design of incubators (experimental incubators were newer, control incubators were older) or slightly different handling procedures could easily have produced the observed difference. The experimental group performed extermely well during the extended salt water challenge period, suggesting that any effect on survival was transient and may have simply been an earlier expression of losses which would have occured in any case.

Control fish performed conparatively poorly during the salt water challenge period. The reasons for this are not entirely clear. It is possible that physiological accommodation to metals through exposure pre-conditioned the experimental group to sudden salt water exposure, since the site of action for some of the metals used (notably Cu and Zn) is at the iono-regulatory level. This might explain the better-than-expected performance of the experimental group, but would not explain the poorer-than-expected performance of the control group. The control group had the same weight and appearance as the experimental group when penned, but began performing more poorly in terms of growth as well as survival early in the salt water phase. Performance of the Gasteneau group was intermediate between the experimental group and the control group for as long as the Gasteneau group was held (30 days). The fact that the control group significantly underperformed the much larger Gasteneau group, although inconclusive, suggests that some specific but unknown factor(s) could be involved in the unusually high control group mortality.

Heavy metal body burden analyses indicate bio-accumulation tendencies which vary among the metals tested. Silver exhibited modest bio-accumulation and a leveling off toward the end of the two week exposure period, with at least two-thirds of the amount accumulated disappearing rapidly upon entry into sea water. The whole body burdens remaining after only a few days in salt water reflected the body burden of the experimental group before the start of the second exposure episode (about 20 ppb), and fell to about half that level by the end of the salt water challenge period. Silver is the only metal tested which was not enhanced in tissues during the salt water challenge period; it was also the only metal tested which was not present in elevated concentrations in the food supply. A portion of the body burden of silver derived from the first or second exposure episode was apparently bound somewhere in the viscera.

Cadmium also exhibited a modest tendency for bioaccumulation in the experimental group and a leveling off toward the end of the exposure period. Some reduction in tissue levels occurred upon entry into sea water, followed by a steady increase. This increase was mirrored by a steady increase in whole body burdens of cadmium in the control group. Cadmium is not deliberately added to the standard regimen, but is present at a concentration about two orders of magnitude greater than the target levels for experimental group incubation water. Hence the elevated tissue concentrations in both experimental and control fish at the end of the salt water challenge period.

Copper showed strong bio-accumulation tendencies in both experimental and control groups during the incubation phase. Whole body burdens in the experimental group leveled off after the first week of exposure. Although copper concentrations in incubation water were well over an order of magnitude higher on the experimental side of the bioassay, control fish exhibited fully 75% of the relatively high whole body burdens found in the experimental fish; differences in whole body burdens between experimental and control groups decreased to insignificance after only a few days in salt water. This strongly suggests an active physiological modulator of copper as indicated by the work of Lauren and McDonald (1986, 1987a, 1987b). Steady increases in whole body burdens of copper for both experimental and control fish after salt water penning reflected the elevated concentration of this metal in the food supply. Tissue concentrations were below those in the food, however, indicating that excessive bio-accumulation was not occurring.

Lead exhibited a moderate tendency for bio-accumulation at exposure levels for the experimental group, and accumulation had not leveled off by the end of the exposure period. Reduction in whole body burdens in experimental fish upon entry into sea water was relatively rapid initially, slowing after 2-3 weeks. High values for both experimental and control groups in whole viscera near the end of the salt water challenge period may be attributed to a relatively high concentration of this heavy metal in the food. This elevated dietary component is undoubtedly also responsible for the reversal in the declining trend in the whole body burden of lead in experimental fish, and the rise in whole body burdens for control fish. Some lead derived from the first or second exposure period appears to have been bound in bone. This deposition of lead in bone combined with the elevated level in the food supply may explain the failure of whole body burdens of experimental and control groups to converge completely.

Zinc showed strong bio-accumulation in both experimental and control fish; whole body burdens did not differ between the two groups throughout the exposure period, in spite of incubation water concentrations more than an order of magnitude higher for the experimental group, and throughout the salt water challenge period. This strongly suggests physiological regulation of zinc at ambient concentrations bracketed by experimental and control incubation water concentrations used in this bioassay. By the end of the salt water challenge period, whole body zinc levels, obviously derived from the food supply, were twice the levels observed in both groups during the exposure period.

On balance, this bioassay was a very successful test of the ability of the Sheep Creek Hatchery to continue unimpaired operations using A-J mine tailings pond water as an interim water supply during transient periods of insufficiency (up to two weeks' duration) due to the presence of the proposed tailings reservoir in the upper Sheep Creek drainage. This test has assumed that deliterious constituents of the tailings pond water would be limited to the five metals chosen for this bioassay (see Appendix) at or below the "target" concentrations, and that incubation water hardness will be adjusted upward to about 300 mg/l as $CaCO_3$ (about 6% sea water). If these assumptions are met, this bioassay indicates that continued successful operation of the Sheep Creek Hatchery can be reasonably expected.

ACKNOWLEDGMENTS

I wish to gratefully acknowledge Echo Bay Exploration, Inc., especially Frank Bergstrom, for encouragement to perform this work and for financial sponsorship. I also wish to acknowledge the extraordinary cooperation of Douglas Island Pink and Chum (DIPAC), especially Ladd Macauley, and the insights and continuous assistance of the Sheep Creek Hatchery Manager, John Ennor. Very special thanks are due to Dr. Terry Mudder of SRK Engineers for his assistance in experimental design and valuable insights into the chemistry of the system. Finally, Columbia Analytical Services, Inc., an independent testing laboratory, is to be congratulated for very rapid turnover of water and tissue samples and excellent quality control.

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PHTHALATES: NON-PRIORITY POLLUTANTS

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Chemical Manufacturers Association
Phthalate Esters Environmental Research Task Group

The Phthalate Esters are considered criterion chemicals in North America. Both Canada and the United States have water quality guidance values for phthalates which were established to protect aquatic life. The current guidance values for acceptable concentrations of phthalates in North America surface waters are all in the low parts per billion range (<4.0 ug/L). These values were based on a body of work from the mid-1970's which, for a number of reasons, has been called into question.

The U.S. EPA, in its recently released "draft" water quality criteria document for Di-2-ethylhexyl Phthalates (DEHP), has chose to regard these early reports of effects at very low (i.e., ppb) concentrations as not consistent with the rest of the literature for phthalates. The U.S. EPA "draft" criteria document suggests that the DEHP water quality value be revised upward from the current 3.0 ug/L to values of 400 ug/L and 360 ug/L for acute and chronic criteria, respectively. These proposed values approximate the maximum water solubilities which have been reported for DEHP (250 to 400 ug/L). Therefore, calling into question the true priority of a class of chemicals whose lead chemical of concern, will not be a significant ecological risk at the maximum aqueous exposure possible.

This presentation will review the scientific and historical basis for suggesting the phthalates be considered a priority of regulation and provide a rational for why the phthalates have historically been over-regulated. A comprehensive set of environmental fate and effects data for 13 commercial phthalates, developed over the last decade by the Chemical Manufacturers Association Phthalate Esters Panel, will be utilized in this effort. The data set includes physical/chemical properties and biodegradation data which have previously been published, plus acute toxicity for 9 aquatic species and a chronic daphnid reproduction data which soon will be presented. These data, as well as other recently published work, will be published.

The toxicity, persistence, and bioaccumulation potential of phthalates are summarized and evaluated within the context of decision criteria for declaring that a chemical be a priority pollutant. Evidence for the inherent metabolism by bacteria and fish suggest that phthalates will neither persist nor significantly bioaccumulate. A case will be made that most phthalate are relatively non-toxic to aquatic organisms in acute exposures and provide no real chronic hazard when historical reports of environmental concentrations are compared with existing chronicity data.

PLATFORM SESSION
Acute vs. Chronic Responses

Chairs: E. Power and K. Munkittrick

Overwintering, Key to 1st Year Largemouth Bass Recruitment in Acidified Northern Waters.

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Largemouth bass in Little Rock Lake, located in north central Wisconsin, have been found to spawn, hatch and grow at pH 5.2; but few, if any, survive their first winter; whereas a new year class is recruited at pH 6.1. Laboratory chronic exposures of y-o-y largemouth bass to different pHs at under-the-ice temperatures (3.8°C)in water of low (13.4 mg/L) and very low (1.5 mg/L) calcium concentration have demonstrated that survival is reduced at pH 4.5; and at pH 4.5 and 5.0 in the softer of the two water types when 30 μg Al/L is added (as monomeric inorganic aluminum). This Al concentration is approximately that of Little Rock Lake when acidified to pH 5.2. Evidence is provided to suggest that osmoregulatory failure is the course of the increased mortality rates observed.

THE INDUCTION OF LETHALITY IN RAINBOW TROUT EXPOSED TO THIOCYANATE BY APPLICATION OF A STRESSOR: ACUTE OR CHRONIC RESPONSE?

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INTRODUCTION

Thiocyanate (SCN^-) is an inorganic anion that may occur in gold-mining effluents (Ingles and Scott, 1987) or be produced by from the metabolic detoxification of HCN by aquatic organisms (Leduc, 1984). As a toxicant, SCN^- competitively inhibits halide movement across biological membranes (Wolff, 1964) and acts specifically as an antithyroid (Yamada et al., 1974). The objective of this paper is to discuss the unique mortality pattern in rainbow trout that is brought about in SCN^- exposed fish by the application of an outside stressor. Due to the rapid onset of mortality, the phrase "Sudden Death Syndrome" (SDS) has been used to describe this condition (Heming et al. 1985). This lethal response appears to be time-independent (up to 16 weeks), with the application of the stressor being the critical factor in the onset of mortality.

MATERIALS AND METHODS

Rainbow trout (*Oncorhynchus mykiss*) were exposed to waterborne SCN^- for durations ranging from 96 h during acute toxicity testing to four months during a chronic toxicity test. During chronic tests, juvenile rainbow trout (3 g) were continuously exposed to nominal waterborne SCN^- levels of 0, 40, 80, 120, or 160 mg/L for 16 weeks. Mortality was monitored daily, and the fish were weighed every two weeks to determine growth rates and to provide a stressor capable of inducing Sudden Death Syndrome (SDS).

RESULTS

Levels of waterborne SCN^- that were not lethal to rainbow trout during a given exposure period became lethal during the application of a physical stressor, such as chasing with a dip net in the tank. Moribundity was rapid, with instantaneous loss of buoyancy and righting reflex, convulsions, rapidly oscillating pigmentation changes, and erratic swimming. This was followed by extreme muscular rigor, with arched backs and flared operculae, and death within minutes.

Mortalities increased with SCN^- concentration, with all fish exposed to 160 mg/L dying by 12 weeks. Approximately 40% of the fish exposed to 120 mg/L were dead by the end of the growth trial. Mortality patterns could be divided into two groups; those exhibiting signs of SDS and those that did not. Sudden Death Syndrome, coincident with the bimonthly weighings, was apparent in varying proportions at the two highest concentrations of SCN$^-$, but was absent in fish exposed to the lower SCN$^-$ levels.

DISCUSSION

Since stress induced mortality in this study appeared to be time-independent, the relevance of the terms acute and chronic in describing this mortality pattern is somewhat obscure. The term acute is often used to describe short-term effects in exposure regimes, usually referring to periods of four days or less (Sprague, 1973). Perhaps acute should also be used to describe rapid or precipitous responses to toxicants, such as in the case of SDS in SCN$^-$-exposed trout. One suggestion would be to avoid the use of acute and chronic to describe responses to toxicants but employ the terms such as "lethal" and "sublethal", with reference to the exact time frame in which responses occur.

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CHRONIC TOXIC EFFECTS OF CYANIDE ON TROPICAL MARINE FISH

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ABSTRACT

The collection of tropical marine aquarium fish using pulse doses of sodium cyanide is described. The collectors cannot control the exposure concentration and cyanide is believed to damage corals and associated fauna. Cyanide toxicity causes about 50% acute mortality on the reef, and >80% of fish collected exhibit delayed chronic mortality from reef to retailer. The Sudden Death Syndrome (SDS) may result from internal damage to the fish caused by the initial uptake of hydrocyanic acid, and due to the slow excretion rate of marines. A procedure using ion selective electrodes was developed to measure cyanide and thiocyanate ion concentrations. Five fish species from Indonesia tested in the USA had cyanide ion concentrations ranging from 5.8 to 23.0 mg/kg. The test is being evaluated through uptake and clearance studies. It can be used to stop the sale and trade of aquarium and food fishes caught with cyanide.

INTRODUCTION

Philippine Situation

While a considerable amount of scientific literature exists concerning the acute and chronic effects of cyanide(s) on freshwater fish, there is almost no scientific information pertaining to the effects of cyanide on marine organisms (Leduc 1981; 1984). The present paper reports information obtained from the aquarium hobby literature and through information gathered by the first author pertaining to the use of sodium cyanide (NaCN) to capture marine aquarium and food fishes in the Philippines (Rubec 1986; 1988a).

In 1983, an American befriended Filipino fish collectors and observed how they used NaCN in plastic squirt bottles for capturing aquarium fishes (Robinson 1983a,b). Several dozen articles published from 1983-1985 revealed the widespread use of cyanide for fishing in the Philippines (reviewed Rubec 1986).

The cyanide fishermen with a "production-at-all-costs" mentality are believed to be contributing to the destruction of Philippine coral reefs and their associated flora and fauna (Cruz 1986; Rubec 1986, 1987a). A controlled study by the Coral Reef Research Team of the Bureau of Fisheries and Aquatic

Resoures found that two applications of cyanide, four months apart, killed corals in test quadrats situated off the Island of Cebu. The coral heads initially took on a bleached appearance, and later became encrusted with algae.

Cyanide tablets the size of hockey pucks are broken apart and inserted into plastic detergent bottles (Rubec 1987a). The collectors use the dissolved hydrocyanic acid (HCN) to kill food fish and to stun aquarium fishes. As a diver approaches, the fish seek refuge in the coral. The fish collector seals off all the exits by squirting clouds of milky HCN solution on the coral head. The concentration of HCN in the bottle is sequentially diluted during the dive. Not being able to control the concentration from the bottle, about 50% of the exposed fish die of acute doses which may range from 5-50 mg/L. The remaining fish become disoriented and flee. Some are driven into gill nets, while other stunned fish are retrieved from the bottom. The collector selects about 10% of the exposed fish taking the colourful species of interest to aquarists. Many of the fish placed in clean seawater appear to recover.

A clandestine trade exists with groupers (lapu lapu) which are smuggled to Hong Kong restaurants (Robinson unpublished MS). At least six 30 m vessels with from 50-80 divers use up to 1250 kg of NaCN for a 10-20 day fishing trip off the Island of Palawan. The Mischief Reef, which was impounded by the Coast Guard, was found to have 30 live wells in the hold, each approximately 1.25 m^2 by 2.5 m deep. The vessels transport 3-5 live tons of lapu lapu directly; while smaller boats transport their fish to Manila, where they are airfreighted to either Hong Kong or Tiawan.

It is estimated that there are about 1500 aquarium fish collectors and possibly another 1500 fishermen in the lapu lapu trade (Rubec 1988a). It is not known how many of the estimated 770,000 fishermen use NaCN to capture food fish. About 800,000 mt of fish are captured by municipal fishermen and 500,000 mt by commercial fishermen. Newspaper reports suggest that 10-20% of the total landings may have been caught with NaCN. Poisons to capture food fish include NaCN, chlorine bleach, and extracts from local roots and seeds, which are directly applied from various containers, or mixed with shrimp or other bait and scattered by hand (del Norte et al. 1989). A larger scale fishing operation was observed from an ultralight aircraft near Bolinao in NW Luzon. A motorized boat was used to spread poison in a large circle up to 100 m across. The dead fish were observed being gathered by more than 20 fishermen operating from bamboo rafts. Blast fishing is often conducted in a similar manner. Both poisoning and blast fishing were stated to be often used in conjunction with circling or drive-in nets such as kayakas and muro-ami fishing. Tiawanese and Japanese vessels have also been reported to use NaCN in Philippine waters.

A study of the sociocultural dynamics of blast fishing and NaCN fishing has been conducted in two fishing villages in the Lingayan Gulf area of NW Luzon (Galvez et al. 1989). It was perceived by those involved in these fisheries that the economic gains derived outweighed the negative consequences to people's health, coastal resource depletion, and the potential penalties associated with law enforcement. The blast fishermen, and other fishermen in the community stated that the main cause of the declining catch was the rampant use of NaCN by the aquarium fish gatherers. The cyanide users stated that cyanide had no harmful effect on the corals or the aquarium fishes sold to exporters.

Through interviews, it has been determined that a trail of death exists with the marine aquarium fishes transported from the reef to pet dealers (Rubec 1987b,c,d). While rates vary, on average about a 30% mortality occurs at each step of the chain from collector, to exporter, importer, retailer, and the marine hobbyist. Hence >80% of the live fish captured die before the remainder are sold by the retailer. In contrast, fishes caught with fine-mesh nets exhibit >90% survival.

The International Marinelife Alliance (IMA) has been training Filipino aquarium fish collectors since 1986 to use fine-mesh (<38 mm) barrier nets and hand nets (Rubec 1988b). Nets cost the collector about $25 (US) per year compared to $400-500 per year to purchase NaCN. Once trained, a net collector can capture as many species and more individual fish per day than the cyanide collector. A network of informants world-wide indicate that net-caught fish are a viable economic alternative to those caught with cyanide because of their higher survival during transport, their vitality, and greater longevity in aquarists' tanks.

Histopathological Studies By Aquarists

Marine aquarium fishes from the Philippines examined at the Steinhart Aquarium in the mid 1960's were found on microscopic examination of histological sections to have necrotic liver tissues (Dempster and Donaldson 1974). There were also histopathological abnormalities of the kidney, spleen, and brain tissues. Experiments were conducted where various marine fish species obtained from California coastal waters were pulse exposed for periods varying from 2.5 to 25 minutes with concentrations of NaCN varying from 1-50 mg/L. The fish were removed to clean water when they started to exhibit signs of distress. The fish which died during the experiments, and the survivers subsequently sacrificed had their tissues examined as described above. All the cyanide exposed fish exhibited gross abnormalities in their liver, kidney, and brain tissues. The kidney and brain tissues also contained excessive amounts of iron. The autopsy findings of the cyanide exposed fish from California were similar to the findings with fishes obtained from the Philippines.

Bellwood (1981a) reported histological damage to the intestine of Dascyllus trimaculatus obtained from east Africa and experimentally pulse exposed to 1 mg/L or to 5 mg/L of NaCN solution for 2-3 minutes. Observation included changes in the external colour of the liver and spleen after 48 h. Cyanide in the stomach resulted in the sloughing of the gastric mucosa, followed by cellular degeneration. The histological damage to the stomach and anterior intestine noted was attributed to the fish swallowing water containing cyanide.

Bellwood (1981b) also exposed specimens of Pomacentrus violascens with 1.84 mg/L of radioactive potassium cyanide ($K^{14}CN$) for 2.5 minutes. The distribution of KCN in various organs was determined by measuring their radioactivity. The tissues with the highest radioactive counts were the spleen (2284 dpm/mg), the gills (1187 dpm/mg), the liver (834 dpm/mg), and the brain (808 dpm/mg). Bellwood proposed that cyanide was rapidly taken up through the gills, with less taken up from the stomach (271-404 dpm/mg). The high counts in the spleen and liver were explained on the basis that these organs concentrate blood-borne cyanide.

The damage to internal organs such as the liver (Dempster and Donaldson 1974) and to the the anterior intestine and stomach (Bellwood 1981a,b) can help to explain why many tropical marine fishes in aquarists' tanks often refuse to eat, or eat but continue to waste away. The destruction of the mucosal cells of the stomach inhibits digestion and assimilation. The liver is important for internal metabolic processes. Damage to the brain may help explain why many marines exhibit lethargic behaviour and often appear to be blind. Chronic delayed mortalities observed with freshwater fish has been termed the Sudden Death Syndrome (SDS) by Heming et al. (1985). Marine fishes exhibit similar symptoms, before dropping dead for no apparent reason in aquarists' tanks several weeks to several months after purchase (Rubec 1986).

Development Of Cyanide Test

Many marine fishes in the trade and in vendors' tanks suspected of being caught with cyanide exhibit symptoms and behaviours which are difficult to conclusively ascribe to cyanide poisoning. Hence there is an urgent need for an objective test to determine cyanide concentrations in the tissues of marine fish. This could be used to regulate the trade in marine aquarium fishes and to assess the use of cyanide in the commercial fisheries. A reliable low-cost test procedure has recently been developed by Dr. R. Soundararajan.

MATERIALS AND METHODS

Theory:

Cyanide levels can be determined fairly accurately by converting cyanide (organically bound or inorganic forms in the fish's body) into water soluble sodium cyanide or sodium thiocyanate, and then estimating the concentrations of cyanide (CN^-) or thiocyanate (SCN^-) ions by ion selective electrodes (ISE).

Procedure: The conversion can be done by grinding preweighed fish in a high speed explosive proof blender with a 10 molar solution of sodium hydroxide for 30 minutes, and then mildly refluxing the ground mixture for an hour. The solid matter can then be decanted/centrifuged, and the supernatant solution made up to a known volume.

The made up aqueous cyanide/thiocyanate solution can be used to determine concentrations of each specific ion using the respective ISE. The ISE should be subjected to a five point calibration using known cyanide or thiocyanate ion concentrations. The respective concentration of each ion can be determined from the unknown solution by measuring the ISE voltages and comparing them with voltages determined from known concentrations on a working graph.

Eleven marine fish specimens representing 9 species were obtained frozen from a New Jersey based pet dealer, who did not divulge their geographic source of origin prior to the tests being conducted. These fish had died for unexplained reasons, and were immediatly frozen in plastic bags. This preliminary testing was conducted in West Plains, Missouri as briefly described above using a CN^- selective electrode.

RESULTS

The marine fishes tested exhibited CN^- levels which ranged from 1120.0 mg/kg for a six inch specimen of Clown Triggerfish from the Philippines, 5.8 to 23.0 mg/kg for five species from Indonesia, to none detected for a Flame Angel from the Marshall Islands, for two French Angels from the Caribbean, and for two species of surgeon fish from Hawaii (Table 1).

The aqueous samples from the fish specimens, which gave positive results with ISE, were also tested using a colorimetric procedure. The blue colour due to the formation of a ferro-cyanate complex (Prussian Blue) confirmed the presence of CN^-. No testing has so far been done to determine whether SCN^- was present in the fish.

Table 1. Cyanide ion concentrations in tropical marine fishes determined using an ion selectiove electrode.

Common name	Scientific name	Geographic source	Concentration CN^- mg/kg
Clown Trigger	*Balistoides conspicillum*	Philippines	1120.0
Bicolor Angel	*Centropyge bicolor*	Indonesia	23.0
Koran Angel	*Pomacanthus semicirculatus*	Indonesia	10.6
Red-tailed Butterfly	*Chaetodon collare*	Indonesia	21.8
Clown Trigger	*Balistoides conspicillum*	Indonesia	5.8
Emperor Angel	*Pomacanthus imperator*	Indonesia	18.6
Flame Angel	*Centropyge loriculus*	Marshall Islands	ND*
French Angel	*Pomacanthus paru*	Caribbean	ND
French Angel	*Pomacanthus paru*	Caribbean	ND
Yellow Tang	*Zebrasoma flavescens*	Hawaii	ND
Naso Tang	*Naso lituratus*	Hawaii	ND

* None Detected

DISCUSSION

Cyanide is known to be used in the collection of marine aquarium fishes in the Philippines, Indonesia, Malaysia, Thailand, Haiti and in the Red Sea off Egypt. The positive results for the fishes tested from the Philippines and Indonesia confirm that cyanide is present. Research is needed to determine whether or not marine fishes have background levels of cyanide, before one can conclude that the positive tests were due to the fish having been collected with cyanide. The negative results for the fishes from the Marshall Islands and Hawaii, where nets are used to capture marine fish support the contention that background CN^- levels are very low or not present. Marine fishes from the Caribbean, with the exception of Haiti, are usually collected with nets, or with anaesthetics such as quinaldine or quinaldine sulfate. The absence of CN^- from the two French Angels tested from the Caribbean support this contention. Further testing is needed before one can obtain an accurate picture concerning the prevalence of the use of cyanide in the Philippines and other countries.

The presence of CN^- at such high concentrations is a surprize. In theory, almost all of the CN^- would be expected to have been converted by the enzyme rhodanese to SCN^- (Leduc 1984). Research on rainbow trout maintained in freshwater indicates that there is a rapid conversion of CN^- to SCN^- (D.G. Dixon personal communication). Perhaps the damage to the liver and other internal organs, where rhodanese is concentrated, is inhibiting the conversion of CN^-.

Further research is presently in progress by the IMA in collaboraton with the Osborn Laboratory at the New York Aquarium. Uptake and release experiments are being conducted to determine the concentrations of CN^- and SCN^- in marine fish over time. One hypothesis being tested is that marine fishes retain cyanide because they have a low urinary excretion rate in comparison to freshwater fish. This research will clarify the rates of uptake and clearance, and the utility of the ISE methodology for monitoring cyanide levels in marine fishes.

The levels of CN^- found with the specimens from the Philippines and Indonesia indicate a potential threat to human health from eating cyanide poisoned fish. In both of these countries fish represents about two thirds of the protein consumed. There have been reports of Filipinos developing stomach cramps and diarrhea after eating fish. The hazards of handling NaCN tablets, diving through clouds of HCN, and consuming cyanide poisoned fish should be evaluated.

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ZINC SENSITIVITY OF *SELENASTRUM CAPRICORNUTUM* IN ALGAL ASSAY MEDIUM WITH VARIOUS EDTA CONCENTRATIONS

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ABSTRACT

The presence of high concentrations of a toxic chemical in solution does not necessarily mean that it will be toxic to an exposed organism. The toxic chemical may be associated with other elements which rendered it unavailable biologically. Environmental complexing can reduce the potential danger of many hazardous substances. Conversely, it is possible that relatively non-toxic single substances may become more toxic in combination. Chemical analysis and toxicological data contain inherent limitations that require a holistic approach be taken in evaluating toxicity in environmental samples. If the difficulties of using chemical analysis alone could be disregarded, the fact remains that chemical analysis of complex environmental samples does not answer the fundamental question of whether a sample is toxic to living organisms. Biologists and chemists must work cooperatively to determine the causes of ecotoxicity.

One choice for a simple toxicity based laboratory bioassay approach to hazardous chemical waste testing with an aquatic plant uses the green algae *Selenastrum capricornutum*. After 96-hours exposure the algal cells in each chamber are counted and the waste concentration (groundwater, surface water, or elutriate prepared from soils and sediments) that causes the chosen end-point effect is calculated. The end-point concentration becomes a quantified measure of the percent of environmental sample that could cause harm to aquatic organisms if exceeded for a particular period of time. It is usually stated as an EC50 and the waste constituents creating the toxicity need not be specifically identified.

Porcella (1983) prepared a "Protocol For Bioassessment of Hazardous Waste Sites", for the U.S. Environmental Protection Agency, which contained both aquatic and terrestrial tests. The protocol described an algal toxicity bioassay that has been utilized at the Environmental Research Laboratory, Corvallis, Oregon for about 11 years. A similar test method for testing of effluents was published by Horning and Weber (1985). Their test, however, described an algal assay dilution medium (AAM) that had been modified by removal of the organic chelator ethylenediaminetetraacetic acid (EDTA). Concerns have been expressed that the Horning and Weber (1985) algal toxicity bioassay: (1) resulted in algal growth that was erratic between replicate cultures (i.e., unacceptable coefficients of variation), and (2) could not achieve the minimum algal cell concentrations required in the acceptability criteria. These growth problems can be attributed to removal of EDTA from

the growth medium.

The presence of EDTA in the inorganic growth medium (AAM) is required to maintain trace nutrients, essential for algal growth, in a biologically available form. The indirect effect of EDTA upon algal growth is through chelation of trace metals in the AAM. Effects of chelation can: (1) lead to increased algal growth by increasing the availability of trace metals; (2) increase growth by decreasing toxic levels of trace metals; (3) reduce growth by decreasing available concentrations of trace metals by formation of strong chelates, and; (4) reduce growth by decreasing concentrations of metals antagonistic to toxic trace metals uptake (Saunders, 1957). Such effects are quite dependent on both the nature and concentration of chelators and trace metals involved. Concern over the potential detoxifying effects of chelation on unknown constituents in effluents and complex wastes, and during the testing of new compounds resulted in decisions to remove EDTA from algal assay medium (AAM) in some algal toxicity bioassays.

Important elements, such as nitrogen and phosphorus, are first order limiting nutrients. When these nutrients are used up by the test algae further increases in biomass stops. On the other hand, the effects caused by interactions between chelators and essential trace nutrients are second order. The required elements are present in adequate quantities, however, they are not immediately available for use by the test algae. Stumm and Morgan (1970) have indicated that the range of concentrations of organic materials in surface waters is 0.1 to 10 mg/L. A study by Barber (1973) produced convincing evidence for the existence of naturally occurring organic ligands that are functionally analogues of synthetic chelators such as EDTA. EDTA, therefore, is a reasonable component of inorganic growth media so long as it is not present in large concentrations excessive to the need for making trace nutrients available to the test algae. The 0.3 mg/L EDTA, which is included in AAM to assure that trace nutrients are not limiting growth, is a conservative quantity when compared to the concentration of synthetic chelator analogues found in nature.

Tests were performed to evaluate the effect of EDTA concentrations on the 96-hour biomass yield of *S. capricornutum*. Eight AAM solutions, that contained all of the required macronutrient and trace metal concentrations (Porcella, 1983) but differed in their EDTA concentration, were prepared. Results from the tests performed in the modified media revealed that their was a linear increase in the 96-hour algal biomass which was related to the increase in EDTA concentrations in the range of 0 to 0.1 mg/L. Algal growth in AAM containing $\leq$0.1 mg/L EDTA was growth rate limited because the trace metal nutrients that were present in the growth media were, depending on the EDTA concentration, partially unavailable biologically. AAM containing 0.1 mg/L EDTA produced 174% more biomass than medium without EDTA. Media containing from 0.1 to 0.3 mg/L EDTA produced similar biomass concentrations after 96-hrs culture. Furthermore, AAM containing 0.3 mg/L EDTA (the original recommended concentration) produced 296% more algal biomass than the medium without EDTA.

Three replicates of each EDTA modified medium were tested. At the conclusion of the tests the coefficients of variation between the replicate control flasks were analyzed. The highest coefficients of variation in medium containing $\leq$0.15 mg/L ranged from 35 to 72 percent, but in some tests they were within 25%. Medium containing 0.20, 0.25, and 0.30 mg/L EDTA produced coefficients of variation that ranged from about 4 to 23 percent. Trace nutrients, readily available for algal growth, appear to improve the reproducibility of results between replicate test flasks and, therefore, the statistical results that support good quality assurance.

From 1982 to 1987 technicians were trained to performed toxicity bioassays using zinc. The tests, performed in AAM containing 0.3 mg/L EDTA, produced an average EC50 concentration of 0.064 mg/L zinc (95% CI = 0.042 to 0.085; r^2 range -0.785 to -0.949). California Gulch Superfund Site samples were tested to determine the EC50 concentrations for percent elutriate. These effect concentrations were converted to zinc using the percent elutriate effect concentrations and the zinc analysis in the parent surface water solutions. The surface waters were diluted with AAM to insured adequate nutrients for the algae, while avoiding chemical effects on nutrient inactivation. The California Gulch sample EC50 concentration for zinc was 0.049 mg/L (95% CI = 0.042-0.059; r^2 -0.907). Leadville Tunnel EC50 concentration was 0.051 mg/L (95% CI = 0.042-0.059; r^2 -0.830). Toxicity measured in California Gulch Superfund Site samples was caused by Zn.

A final set of tests were performed by diluting California Gulch and Leadville Tunnel surface waters with AAM that was prepared without EDTA. The California Gulch sample produced an EC50 of 0.005 mg/L Zn (95% CI = 0.000-0.016; r^2 -0.591) while the Leadville Tunnel result was 0.036 mg/L Zn (95% CI = 0.029-0.046; r^2 -0.765). The Zn concentration in the Arkansas River control station was 0.006 mg/L.

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METALLOTHIONEIN - COPPER RELATIONSHIPS AS AN INDICATION OF CHRONIC STRESS IN RAINBOW TROUT FROM VANCOUVER ISLAND, BRITISH COLUMBIA, CANADA

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Metallothionein and cellular copper concentrations in rainbow trout (Oncorhynchus mykiss) exposed to sub-lethal copper concentrations resulting from acid mine drainage, were compared to populations from three control lakes. Relationships between membrane copper, cytosol copper, metallothionein copper, metallothionein, and gill histology as they relate to sublethal or chronic toxicity, and existing ambient water quality criteria are discussed. A protocol for using physiological indicators as a test for chronic stress in fish is proposed.

INTRODUCTION

Acid mine drainage is a serious problem in British Columbia because of the potential input of metals from the waste rock and tailings for several thousands of years after the mine has closed. Traditional monitoring methods include the collection of water samples, bioassays, and animal (or plant) tissue measurements to determine the level of toxicity. The bioassay methods have been good methods of measuring acute toxicity, however, chronic toxicity, which includes reproductive or growth effects, are less clear cut. Chronic toxicity has been traditionally determined through the collection of water and tissue samples and comparison to a criteria or objective. In some cases, long term mortality experiments (21-28 days) have been completed to establish chronic mortality (e.g., Deniseger, unpublished).

The problem with the traditional methods is that they are expensive in terms of time and laboratory expenses, and they are an indirect method of determining chronic stress. Biomonitors have the potential to be more effective as they integrate the water quality during events that may have been missed by discrete sampling, or monitor contaminants at concentrations below existing analytical methods.

Biochemical techniques have been developed to determine the significance of contaminants in the environment. Specifically, Roch and McCarter (1984a and 1984b) used hepatic metallothionein levels in the liver of rainbow trout to determine the safe levels of copper, cadmium and zinc in Buttle Lake, B. C. Deniseger et al. (1990) has successfully used the levels set by Roch and McCarter to monitor rainbow trout in Buttle Lake following extensive reductions in metal concentrations from the mine-site.

In addition to metallothionein, copper analysis of the high and low molecular weight protein pools was used by Roch et al. (1982) to differenciate the copper binding sites within the cell. Imber et al. (1987) plotted membrane bound copper *vs* total protein copper, and related the change in gradient with the induction of a specific metal binding protein (e.g., metallothionein).

The purpose this paper is to summarize metallothionein and biochemical data for rainbow trout from Buttle Lake, the Tsolum River watershed, and background sites, to test the observations of Imber et al.

(1987), and quantify the metallothionein and membrane and cytosolic copper fractions with ambient copper concentrations. In addition, the the measured chronic responses will be compared to the present water quality criteria for copper, and a sampling and analytical protocol for metallothionein and biochemical monitoring is proposed.

MATERIALS AND METHODS

The sample locations were a single site on four lakes (Buttle, Maxwell, Old Wolf, and Stocking) and six sites in the Tsolum River watershed. All sites are located on Vancouver Island, except Maxwell Lake which is located adjacent to Vancouver Island, on Saltspring Island (Figure 1).

Tables 1 and 2 summarizes the morphology of the lakes, and some stream characteristics of the Tsolum River sites.

The geology of Vancouver Island and Saltspring islands are a complex mixture of igneous and sedimentary rock. Rainfall is quite variable from site to site. Despite these differences in geology and rainfall, all sites are considered to have soft-water. The general water chemistry for each site is summarized in Table 3.

Site Descriptions:

T1: Tsolum River at Duncan Main: Represents background water quality for the Tsolum River and serves as the control. Located upstream of minesites's influence. Ministry of Environment site: E206513.

T2: Murex Creek at Duncan Main: This site is known to be acutely toxic to salmonids during freshet, and represents the most contaminated site. Ministry of Environment site: E206499.

T3: Tsolum River 500 m downstream of Murex Creek. This site is known to be acutely toxic to salmonids during spring freshet, although copper concentrations slightly lower than site T2. Ministry of Environment site: E206826.

T4: Tsolum River at Farnham Road. This site is located 10.5 km downstream of Murex Creek. It represents the transition between acute and chronic toxicity. Ministry of Environment site: 0127620.

T5: Tsolum River upstream of Puntledge River. This site is located 13.5 km downstream of Murex Creek. Copper concentrations were well below acutely toxic levels. Ministry of Environment site: 0127621.

T6: McKay Creek at Rossiter Mainline. This site is located just upstream of Murex Creek. It receives low levels of copper from the abandoned Environment site:

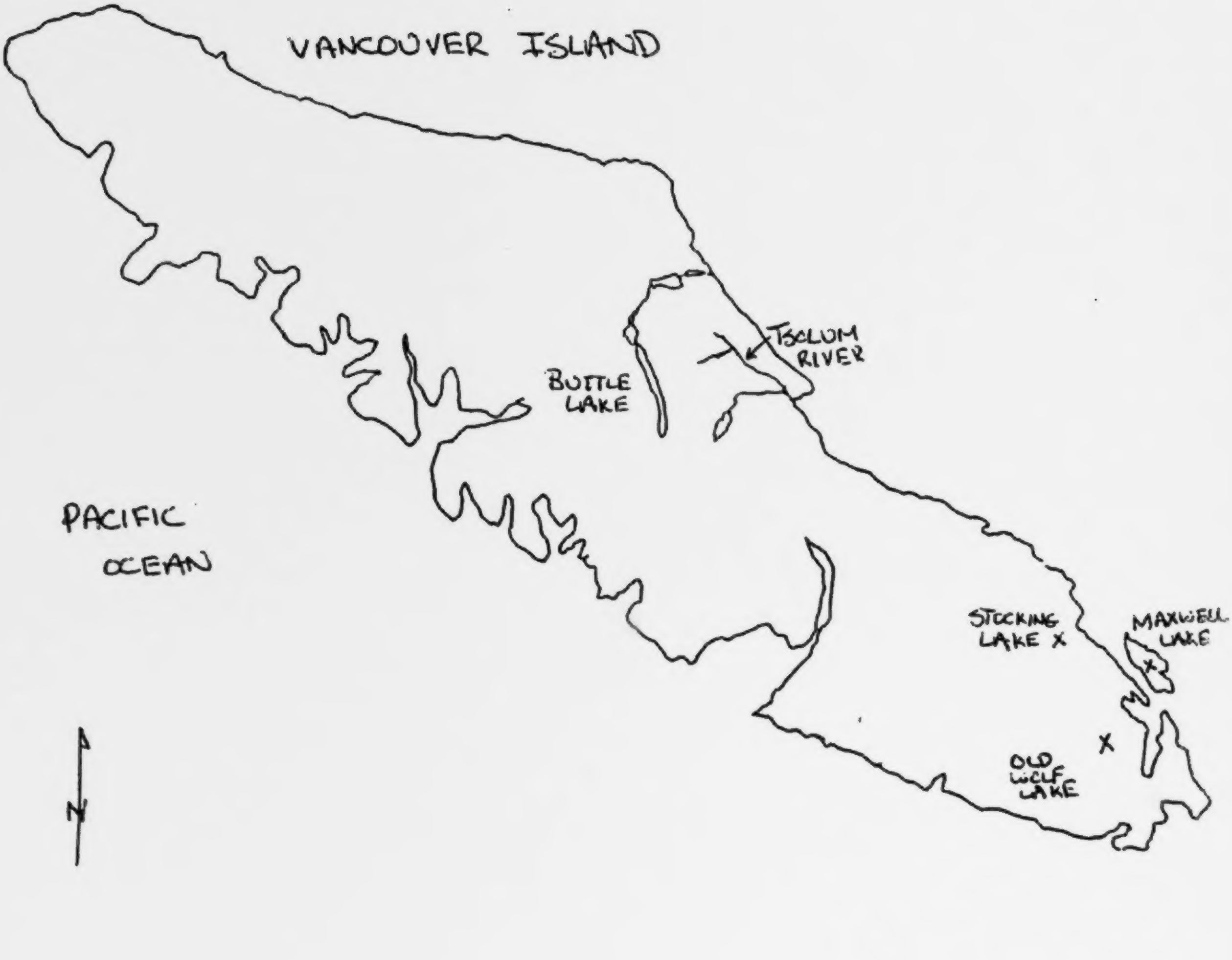

VANCOUVER ISLAND
TSOLUM RIVER
BUTTLE LAKE
PACIFIC OCEAN
STOCKING LAKE X
MAXWELL LAKE
X
OLD WOLF LAKE
X
N

FIGURE 1: STUDY SITES ON VANCOUVER ISLAND

M: Maxwell Lake is a small pristine lake on Saltspring Island. It used as a domestic water supply and has a restricted access. It is monitored monthly for water chemistry as part of an acid rain monitoring program. Ministry of Environment site: 1130022.

OWL: Old Wolf Lake is a small pristine lake on southern Vancouver Island. It is located in the Victoria Water Supply watershed. It is monitored monthly for water chemistry as part of an acid rain monitoring program. Ministry of Environment site: 1130644.

S: Stocking Lake is a small pristine lake on Vancouver Island. It is used as source of domestic water for the City of Ladysmith. It is monitored monthly for water chemistry as part of an acid rain monitoring program. Ministry of Environment site: E206290.

B: Buttle Lake is a large lake near the centre of Vancouver Island. The sample location is at the extreme south end near a copper-lead-zinc mine. Ministry of Environment site: 0130082.

Table 1
Morphology of Study Lakes

Lake	Lake Area (ha)	Maximum Depth (m)	Average Depth (m)	Flushing Rate (-yr)
Buttle	3530.0	125	45.0	1.0
Maxwell	28.0	17	6.5	0.7
Old Wolf	24.0	13	4.4	2.2
Stocking	20.0	26	8.4	2.1

Table 2
Stream Characteristics of the Tsolum River

Site	Wetted Width (m)	Maximum Depth (m)	Average Depth (m)
T1-Duncan	10	0.8	0.8
T2-Murex Cr	12	1.3	0.8
T3-d/s Murex	15	1.0	1.0
T4-Farnham	15	1.0	1.0
T5-Puntledge	15	1.0	0.8
T6-McKay Cr	8	1.5	0.5

Table 3
General Water Chemistry of the Study Sites

Site	pH	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Na^+ (mg/L)	HCO_3^- (mg/L)	SO_4^{2-} (mg/L)	Cl^- (mg/L)
T1-Duncan	7.1	4.0	1.2	1.0	15.9	1.3	3.2
T2-Murex Cr.	6.7	2.8	0.5			5.0	
T3-d/s Murex	6.8	3.1	0.6			4.8	
T4-Farnham	7.1	3.3	0.7	1.0	12.9	3.5	3.2
T5-Puntledge	7.0	3.9	1.0	1.0	14.6	2.1	3.2
T6-Mckay Cr	6.8	2.8	0.6			6.5	
Buttle	7.4	10.0	0.7	0.9	23.2	7.5	3.3
Maxwell	7.3	5.3	1.3	2.8	17.1	4.5	4.8
Old Wolf	7.2	2.0	0.7	1.9	7.8	0.7	3.4
Stocking	7.5	3.8	0.6	1.2	11.4	2.0	2.3

Experimental Design:

Tsolum River: Steelhead smolts (150-180 grams) and coho underyearlings (110-130 grams) were carefully transported from the Puntledge River Salmonid Enhancement Hatchery located on Vancouver Island to six sites on the Tsolum River (Figure 2). Ten coho were deployed in cages at six sites for 28 days (May 1 to May 29, 1990) to determine long term survival. At the termination of the experiment, five surviving coho were sampled for gill histology. Sites were visited a minimum of twice per week. During each visit, water samples for total metals were taken, the fish were fed and checked for mortality and general health. Additional weekly water samples consisting of total metals, pH, and other parameters were also taken at most sites. At the end of the exposure period all live fish were weighed and measured. The fish were caged in a 45 litre fine meshed enclosure, at a density of 40 grams/litre.

To minimize any potential handling stress during the extraction of the coho gills' for histology, the coho were removed from the cages and immediately anaesthetised in MS222 (75 mg/L). After several minutes, the coho were transferred to a lethal solution of MS222 (150 mg/L). The coho gills were blind coded, and fixed in Davidson's solution. The samples were shipped to the British Columbia fish health laboratory in Nanaimo, B. C., where the gills were sectioned at 4 to 6 microns and stained with H and E. The sections were then examined for stress responses as indicated by hypertrophy of the epithelial cells.

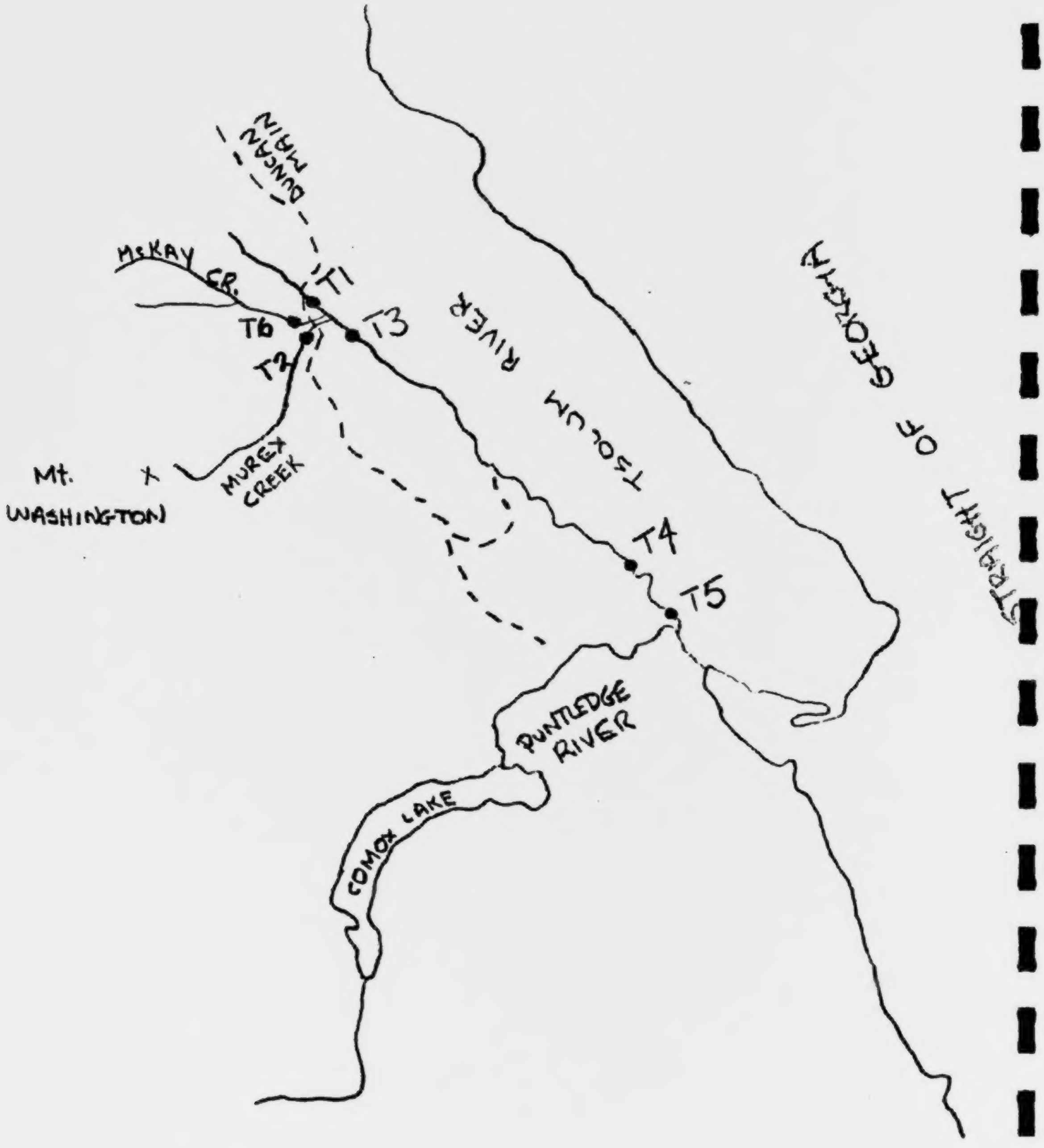

DUNCAN MAIN
McKAY CR.
T1
T6
T3
T2
TSOLUM RIVER
STRAIGHT OF GEORGIA
Mt. WASHINGTON
MUREX CREEK
T4
T5
PUNTLEDGE RIVER
COMOX LAKE

FIGURE 2: WATER QUALITY SITES ON THE TSOLUM RIVER.

Five steelhead trout were placed in cages at 4 of the 6 sites. The sites at Murex Creek at Duncan Mainline (T1) and Tsolum River 500 m d/s of Murex Creek (T2) were not used because of the high copper concentrations and an anticipated high mortality rate. At the termination of the experiment, all surviving steelhead were sacrificed, weighed, and the extracted liver placed immediately on dry ice. Samples were submitted to C.B.R. International for biochemical analysis (see below).

Native rainbow trout were collected during August and September, 1989 from Buttle, Maxwell, Old Wolf, and Stocking lakes, and August 1990 from Buttle Lake. All fish were live caught with monofilament nets or angling. Livers were extracted and stored on dry ice until delivered to the laboratory for biochemical analysis.

Laboratory Analysis:

Livers from live caught fish were extracted, placed in homogenization buffer and placed immediately on dry ice. Samples were shipped frozen to C.B. Research Corporation for analysis. The samples were slowly thawed, homogenized, and an aliquot was withdrawn for dry weight determination by lyophilization. The remaining homogenate was centrifuged at 4°C, 20,000xg for 30 minutes to separate the cytosol fraction from the particulate/membrane-pellet fraction. The membrane fraction was digested and analyzed for copper by flame atomic absorption spectrophometry (FAAS) on a Varian 475. The supernatant was divided into two aliquots, one for cytosol copper analysis by FAAS, and the other for metallothionein analysis.

The metallothionein aliquot was denatured by mixing a 1:1 (w:w) ratio with 95% ethanol for 2 hours and centrifuging 15 minutes (4°C) at 20,000xg. The resulting denatured supernatant was assayed for copper (a measure of copper associated with metallothionein) by FAAS, and metallothionein proteins as determined by polarography as described by Imber et al., (1987).

The total copper concentration of the liver sample was determined by two methods. An aliquot of the homogenized liver prior to centrifugation was digested and analyzed using FAAS for total copper. The second method involved the addition of the copper concentrations associated with the cytosol and membrane fractions.

Copper complexing capacity was determined through the titration of a known volume of sample with a copper stock solution (10^{-4} or 10^{-5} M).

The titration process was controlled by the 646 VA Processor of the Metrohm Polograph as described by C. B. Research (1990a).

Quality control and assurance of the laboratory procedures involved several techniques. Homogenization buffer blanks for metal analyses were subjected to the same treatments as the fish livers, and run concurrently with the samples. The Standard Reference Material, DOLT-1, was digested with the membrane samples to test the efficiency of the digestion procedure for metals extraction. Metallothionein was calibrated using Rabbit metallothionein Type 11(Sigma Chemicals) prepared in homogenization buffer. Each metallothionein result was duplicated, and the average result reported.

Analytical error for all tests was estimated by analyzing two liver samples from the Tsolum River in triplicate. All fish livers collected in 1989 from Buttle Lake, Maxwell, Stocking, and. Old Wolf Lake were analyzed in triplicate.

RESULTS

Extraction of copper from the DOLT-1 Standard Reference Material yielded one analysis at 75% and two analyzes at 88% recovery (C. B. Research, 1990b). The analytical variability determined from triplicate analyses showed precision was better than 10% for the protein determinations. Metal determinations were more variable depending on the copper concentration in the liver. Coefficients of variation ranged from a low of 1-4% for values of >200 µg/g dw, to 10-18% for values between 2-10 µg/g dw.

Ambient copper concentrations at the time of sampling ranged from a low of L1 µg/L at Maxwell, Stocking, and Old Wolf lakes to a high of 50 µg/L at site T2 (Table 4). Copper concentrations in Maxwell, Stocking, and Old Wolf lakes ranged from a high of 1 µg/L during the winter to L1 µg/L during the summer months. Total and dissolved copper levels in the surface waters of south Buttle Lake typically range from L1 µg/L in the late summer to 5 µg/L in the spring (period of maximum runoff). The average for the south Buttle Lake in 1989 was 2 µg/L for both total and dissolved copper. Other metals in the Buttle system were zinc (average=26 µg/L, range=8-30 µg/L), and cadmium (average=0.15 µg/L, range=0.1-0.3 µg/L). At the time of fish sampling the dissolved copper, and zinc concentrations were 3 and 25 µg/L, respectively.

Total copper concentrations in the Tsolum River varied from a low of 3 and 4 µg/L respectively at the Tsolum River near Duncan Main and McKay Creek. These sites are upstream of the acid mine drainage, and represent background copper levels for the system. Average total copper concentrations increased to a maximum of 50 µg/L in Murex Creek.

Table 4
Copper Concentrations and Mortality Data for the Study Sites

Site	Total Copper (μg/L)	Dissolved Copper (μg/L)	CCC* (μg/L) (n=1)	Steelhead Mortality (%)	Coho Mortality (%)
T1-Duncan	3±3 (n=9)	----	24.8	0	20
T2-Murex Cr.	50±13 (n=8)	45±21(n=2)		N/A	100
T3-d/s Murex	35±12 (n=9)	----		N/A	80
T4-Farnham	21±12 (n=8)	14±4 (n=4)	20.3	0	50
T5-Puntledge	14±14 (n=9)	----	34.3	20	40
T6-McKay Cr	4±2 (n=5)	----		damaged	damaged
Buttle Lake	2±2 (n=5)	2±2 (n=5)		N/A	N/A
Maxwell Lake	L1±1 (n=5)	L1±1 (n=5)	27.3	N/A	N/A
Old Wolf Lake	L1±1 (n=5)	L1±1 (n=5)	14.6	N/A	N/A
Stocking Lake	L1±1 (n=5)	L1±1 (n=5)	24.1	N/A	N/A

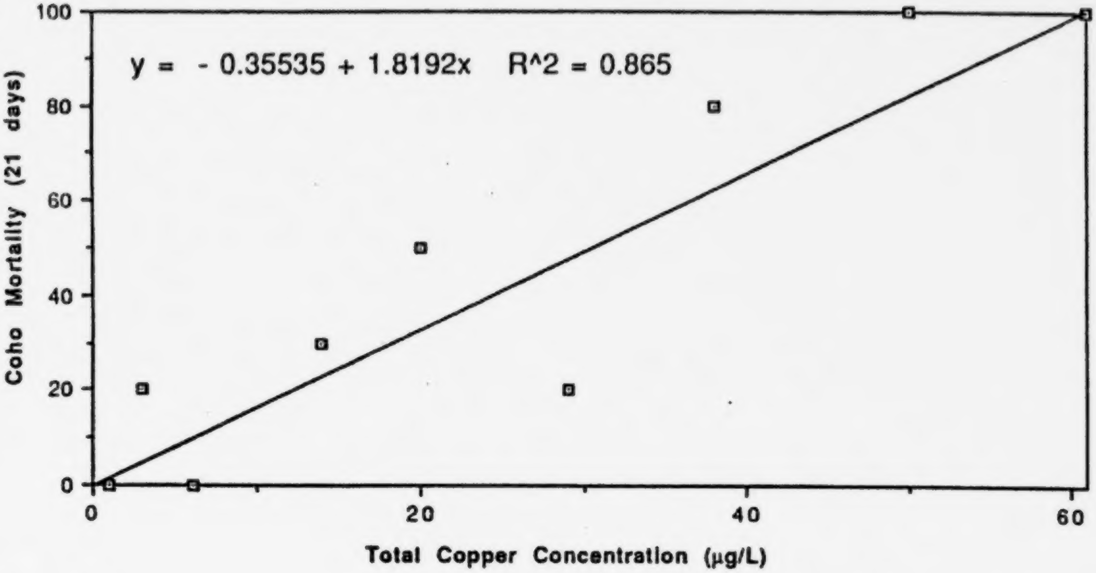
* CCC= Copper complexing capacity

The steelhead and coho cages at McKay Creek in 1990 were damaged after two weeks due to a flood event. Unfortunately, the experiment at this site, could not be continued. No coho or steelhead mortalities were recorded at this site in the first two weeks of the experiment.

The long term coho mortality in the Tsolum River was completed in 1989 and 1990. Mortality was strongly correlated with the average total copper concentration in the Tsolum River (Figure 3). Although this relationship would only apply to the Tsolum River (because of its specific water quality), it is important to note that all sites had no mortality using the 96h LC_{50} criteria. Consequently, all stations were considered to be acutely-nontoxic, however, chronic toxicity was clearly demonstrated.

Results of gill histology on coho from the Tsolum River at Duncan Main, upstream Puntledge River, and at Farnham Road show some associations with the increased copper concentrations (Table 5). Where a sample is listed under a mixed category such as mild-moderate, this denotes that the majority of the tissue was mild, but that there were also moderately affected sites in the gills.

Figure 3: Coho Mortality as a Function of Copper Concentration in the Tsolum Watershed (1989-1990)



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T3-d/s Murex	35±12 (n=9)	----		N/A	80
T4-Farnham	21±12 (n=8)	14±4 (n=4)	20.3	0	50
T5-Puntledge	14±14 (n=9)	----	34.3	20	40
T6-McKay Cr	4±2 (n=5)	----		damaged	damaged
Buttle Lake	2±2 (n=5)	2±2 (n=5)		N/A	N/A
Maxwell Lake	L1±1 (n=5)	L1±1 (n=5)	27.3	N/A	N/A
Old Wolf Lake	L1±1 (n=5)	L1±1 (n=5)	14.6	N/A	N/A
Stocking Lake	L1±1 (n=5)	L1±1 (n=5)	24.1	N/A	N/A

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None of the coho caged in the Tsolum River were observed to have normal gill structure. It is unclear if this was the result of damage at the Puntledge fish hatchery prior to transportation, the transportation, caging, or the consequence of the copper concentrations in the Tsolum River.

With these limitations in mind, the histology results from the site with the lowest copper concentrations (Tsolum River near Duncan Main) had the lowest impacts. Two gills had normal to mild impacts, one had mild impacts, and two had mild to moderate impacts. Gills from caged fish upstream of the Puntledge River ranged from mild (1), moderate (3), and moderate to severe (1). Fish from the Farnham Road site had a wide range of histological results: normal to mild (1), mild to moderate(1), moderate (1), moderate to severe (1), and severe (1). Although no clear pattern exists between gill histology. it is appears that the stations with higher copper concentrations (Puntledge and Farnham) had increased gill damage.

Table 5
Coho Gill Histology Data (1989) from the Tsolum River Watershed

Normal	Normal-Mild	Mild	Mild-Moderate	Moderate	Moderate-Severe	Severe
	Duncan	Duncan	Duncan	Farnham	Farnham	Farnham
	Duncan	Puntledge	Duncan	Puntledge	Puntledge	
	Farnham		Farnham	Puntledge		
				Puntledge		

The biochemical liver analysis involved the determination of metallothionein, and the copper concentrations associated with the cytosol fraction, the pellet or membrane fraction, and the metallothionein like proteins. All results have been reported by C.B. Research (1990a and 1990b) and are summarized in Table 6.

Table 6
Mean and Standard Deviations for Rainbow Trout Livers Collected from 7 Sites on Vancouver Island

Site	Sample Size	Metallothionein (nmole/g dw)	Cytosol Copper (µg/g dw)	Membrane Copper (µg/g dw)	Metallo-Copper* (µg/g dw)	Total Copper (µg/g dw)
Buttle Lake	5	306 ± 176	567±224	248±116	128± 86	1479±629
Maxwell Lake	10	92 ± 27	234±168	161±130	24± 12	----
Old Wolf Lake	9	181 ± 44	448±322	411±292	35± 15	----
Stocking Lake	10	109 ± 23	72± 98	21± 33	18± 10	----
T1-Duncan	5	100 ± 20	223± 50	102± 33	24± 8	491± 112
T5-Puntledge	4	166 ± 37	245± 72	79± 17	48± 13	563± 218
T4-Farnham	5	215 ± 86	360± 89	81± 23	67± 25	683± 160

Total copper concentrations were only determined for Buttle Lake and the Tsolum watershed sites (Table 6). Total copper concentrations ranged from a high of 1479 µg/g (dw) at Buttle Lake to 491 µg/g (dw) at the Tsolum River at Duncan Main. Although the sites from the Tsolum watershed and Buttle Lake cannot be compared (because caged fish cannot be compared to native fish because the length of exposure is different), historical total copper determinations in rainbow trout from Buttle Lake have not reflected the copper concentrations of the water. Total copper concentrations in Buttle Lake between 1966 and 1973 averaged 529±453 µg/g (dw). They did not increase between 1979 and 1981 (539±124 µg/g dw) when the copper concentrations were the highest, or decrease between 1983 and 1986 (510±307 µg/g) after acid mine drainage was reduced to the lake and the copper concentrations decreased (Deniseger, unpublished).

In contrast, hepatic metallothionein concentrations in Buttle Lake have declined steadily in response to decreasing ambient copper concentrations (Deniseger, 1990). Metallothionein concentrations reached a maximum of 1345±115 nmoles.g (dw) in 1981, and have declined to 320±110 nmoles/g (dw) in 1985.

The metallothionein concentrations in rainbow trout ranged from a low of 92 nmoles/g (dw) at Maxwell Lake to a high of 306 nmoles/g (dw) at Buttle Lake (Table 6). The concentrations in the pristine lakes ranged from 92 to 181 nmoles/g (dw). The results from the Tsolum River watershed showed increased metallothionein concentrations with increasing ambient copper concentrations, however, the levels were not significantly higher than the pristine lakes. A longer term of exposure (i.e. >28 days) for fish in the Tsolum watershed may have yielded higher metallothionein concentrations.

The copper associated with the metallothionein proteins showed a similar pattern (Table 6). The highest average concentration (128 µg/g dw) was recorded at Buttle Lake, and the lowest (18 µg/g dw) at Stocking Lake. The average concentration from the pristine lakes ranged from 18-35 µg/g (dw). As expected the results from the Tsolum River showed increasing concentrations with increasing ambient copper concentrations.

Cytosol and membrane copper concentrations were highly variable (Table 6), ranging from a low of 72 and 21 µg/g (dw) at Stocking Lake, to an high of 567 µg/g dw (Buttle Lake) and 411 µg/g dw (Old Wolf Lake). The cytosol concentrations at the Tsolum River sites increased with increasing ambient copper concentrations. In contrast, the membrane concentrations for the same sites appeared to decrease with increasing ambient copper concentrations.

Regression analyses were performed on the hepatic biochemical data and are summarized in Tables 7-14.

Table 7: Correlation Matrix for Rainbow Trout Collected from all Sites (n=68)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC[1]	Total Copper	Metallothionein
Cytosol-Cu	- - - -				
Membrane-Cu	0.82*	- - -			
TPCC	0.96*	0.94*	- - - -		
Total-Cu	- - - -	- - - -	- - - -	- - - -	
Metallothionein	0.22	0.00	0.30	- - - -	- - - -
Metallo-Cu**	0.41*	0.10	0.14	- - - -	0.87*

[1] Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper associated with metallothionein like proteins

Table 8: Correlation Matrix for Rainbow Trout Collected from Buttle Lake (n=10)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC[1]	Total Copper	Metallothionein
Cytosol-Cu	- - - -				
Membrane-Cu	0.98*	- - - -			
TPCC	0.99*	0.99*	- - - -		
Total-Cu	0.99*	0.98*	0.99	- - - -	
Metallothionein	-0.49	0.20	-0.51	-0.22	- - - -
Metallo-Cu**	-0.10	-0.23	-0.11	0.22	0.72

1 Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper associated with metallothionein like proteins

Table 9: Correlation Matrix for Rainbow Trout Collected from Maxwell Lake (n=10)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC[1]	Total Copper	Metallothionein
Cytosol-Cu	- - - -				
Membrane-Cu	0.74	- - - -			
TPCC	0.95*	0.91*	- - - -		
Total-Cu	- - - -	- - - -	- - - -	- - - -	
Metallothionein	0.24	0.22	0.25	- - - -	- - - -
Metallo-Cu**	0.67	0.42	0.60	- - - -	0.75

1 Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper associated with metallothionein like proteins

Table 10: Correlation Matrix for Rainbow Trout Collected from Old Wolf Lake (n=9)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC[1]	Total Copper	Metallothionein
Cytosol-Cu	- - - -				
Membrane-Cu	0.94*	- - - -			
TPCC	0.99*	0.98*	- - - -		
Total-Cu	- - - -	- - - -	- - - -	- - - -	
Metallothionein	-0.71	-0.59	-0.66	- - - -	- - - -
Metallo-Cu**	0.41	0.46		- - - -	0.24

1 Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper associated with metallothionein like proteins

Table 11: Correlation Matrix for Rainbow Trout Collected from Stocking Lake (n=10)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC[1]	Total Coppe	Metallothionein
Cytosol-Cu	----				
Membrane-Cu	0.99*	----			
TPCC	1.00*	0.99*	----		
Total-Cu	----	----	----	----	
Metallothionein	0.21	0.17	0.20	----	----
Metallo-Cu**	0.94*	0.92*	0.94*	----	0.43

1 Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper associated with metallothionein like proteins

Table 12: Correlation Matrix for Rainbow Trout Collected from the Tsolum River at Duncan Main (n=5)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC^1	Total Copper	Metallothionein
Cytosol-Cu	- - - -				
Membrane-Cu	0.65	- - - -			
TPCC	0.95*	0.85	- - - -		
Total-Cu	0.48	0.61	0.62	- - - -	
Metallothionein	-0.89	-0.36	-0.76	-0.32	- - - -
Metallo-Cu**	-0.39	-0.05	-0.29	0.48	0.66

1 Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper concentrations associated with metallothionein

Table 13: Correlation Matrix for Rainbow Trout Collected from the Tsolum River u/s Puntledge River (n=4)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC^1	Total Copper	Metallothionein
Cytosol-Cu	- - - -				
Membrane-Cu	0.84	- - - -			
TPCC	0.99*	0.89	- - - -		
Total-Cu	0.93	0.59	0.88	- - - -	
Metallothionein	0.66	0.16	0.58	0.89	- - - -
Metallo-Cu**	0.74	0.48	0.71	0.68	0.59

1 Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper associated with metallothionein like proteins

Table 14: Correlation Matrix for Rainbow Trout Collected from the Tsolum River at Farnham Road (n=5)

Parameter	Cytosol-Cu	Membrane-Cu	TPCC^1	Total Copper	Metallothionein
Cytosol-Cu	- - - -				
Membrane-Cu	-0.97*	- - - -			
TPCC	0.99*	0.95	- - - -		
Total-Cu	0.79	-0.84	0.77	- - - -	
Metallothionein	0.98*	-0.99*	0.97*	0.87	- - - -
Metallo-Cu**	0.60	-0.63	0.59	0.95*	0.68

1 Total Protein Copper Concentration (cytosol + membrane)

* Significance at 0.01 probability

** Copper associated with metallothionein like proteins

There are many significant correlations in Tables 7-14. Of particular interest is the significant positive relationship between metallothionein and the other biochemical parameters recorded at the station with the highest ambient copper concentration (Tsolum River at

Farnham Road). The correlation coefficient for the Tsolum River upstream of the Puntledge River was also positive, however, the correlation was not significant because of a low sample size. The lack of correlation between metallothionein and the other biochemical tests from Buttle Lake suggests that the ambient copper concentrations were not sufficiently high to have caused the induction of the protein.

Several methods have been used to interpret the relative changes in copper concentration between the cytosolic and membrane bound fractions. Imber et al. (1987) noted that changes in the ratio between membrane and cytosolic copper concentrations were related to the induction of a specific metal binding protein (e. g., metallothionein).

The total protein copper concentrations (TPCC) were estimated by adding the membrane and cytosolic copper concentrations. The correlations (r) between TPCC and cytosolic copper concentrations were consistently high and statistically significant at all sites. The correlations ranged from a low of 0.95 at the Tsolum River near Duncan Main, to 1.00 at Stocking Lake.

The regression line for TPCC and cytosolic copper in the pristine lakes showed two distinct trends: they passed through the origin, and the partitioning of the copper between the membrane and cytosolic fractions was similar (Figures 4 and 5). The exception was Stocking Lake (Figure 6). The slopes for cytosol and membrane both went through the origin (indicating a pristine situation), but the slopes were significantly different. The low concentrations of membrane copper caused the difference in slopes. Further samples are required from Stocking Lake to determine the significance of the low membrane copper concentrations.

The regression lines from the sites with varying copper concentrations were different from the pristine lakes in two ways: firstly, the cytosol-TPCC relationship did not go through the origin indicating that at low concentrations, the majority of the TPCC would be in the cytosol fraction (Figures 7-10). Secondly, the slopes increased with increased ambient copper concentrations (Table 15).

Figure 4: Hepatic Cytosol and Membrane Copper Concentrations as a Function of TPCC in Rainbow Trout from Maxwell Lake

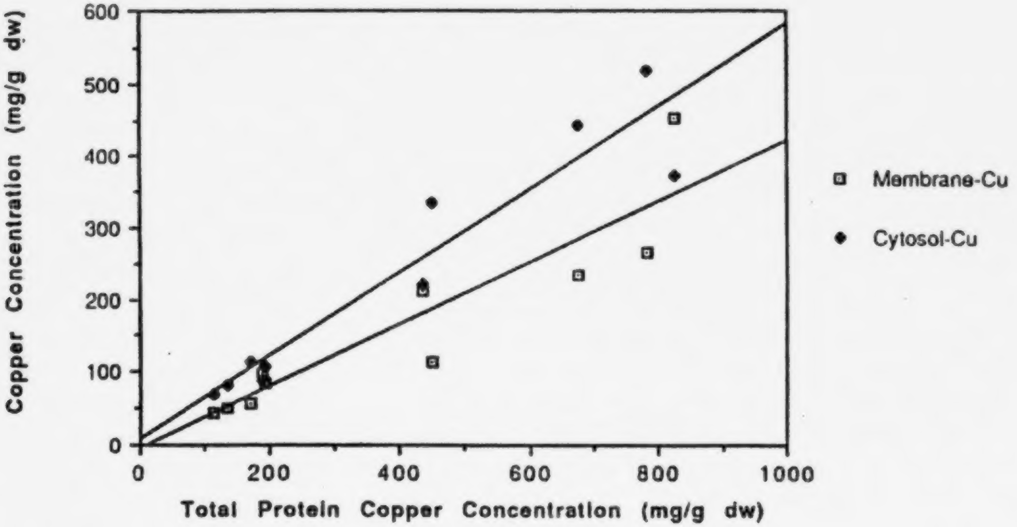


Figure 5: Hepatic Cytosol and Membrane Copper Concentrations as a Function of TPCC for Rainbow Trout from Old Wolf Lake

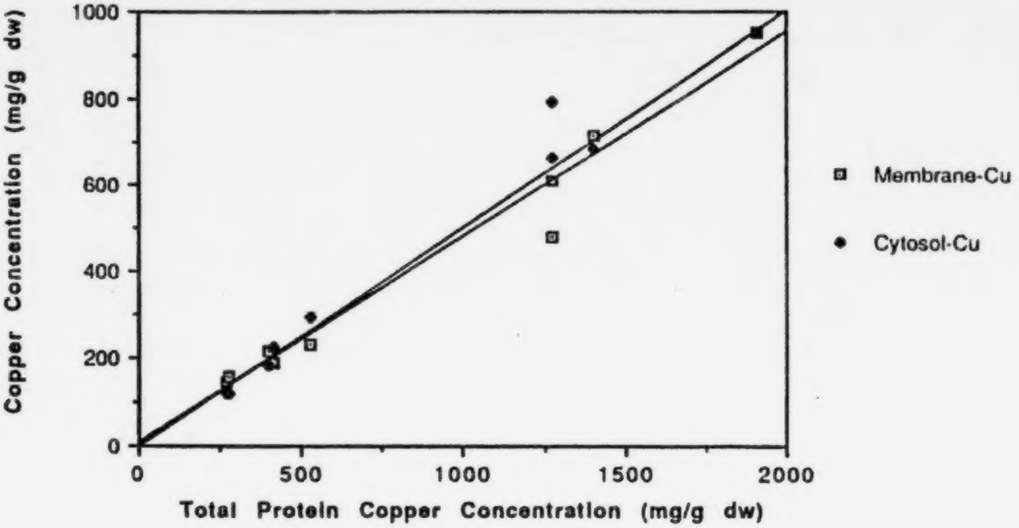


Figure 6: Hepatic Cytosol and Membrane Copper Concentration as a Function of TPCC for Rainbow Trout from Stocking Lake

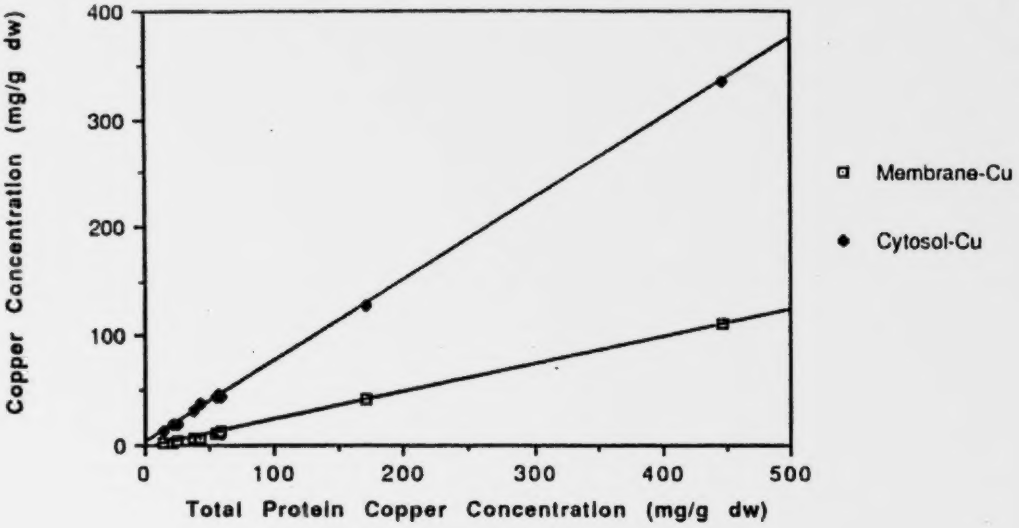


Figure 7: Cytosol and Membrane Copper Concentrations as a Function of The Total Protein Copper Concentrations for Rainbow Trout from Buttle Lake

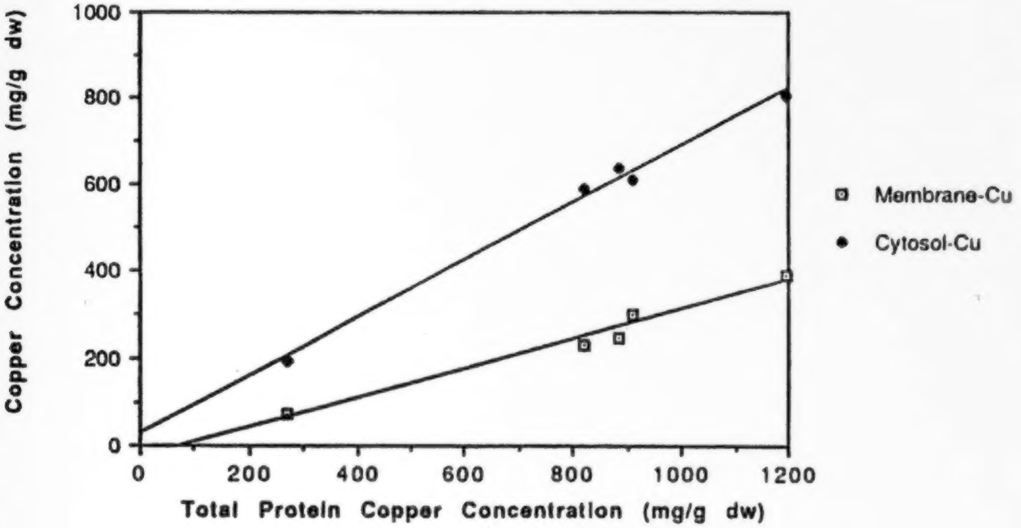


Figure 8: Hepatic Cytosol and Membrane Copper Concentrations as a Function of TPCC for Caged Rainbow Trout from the Tsolum River near Duncan Main

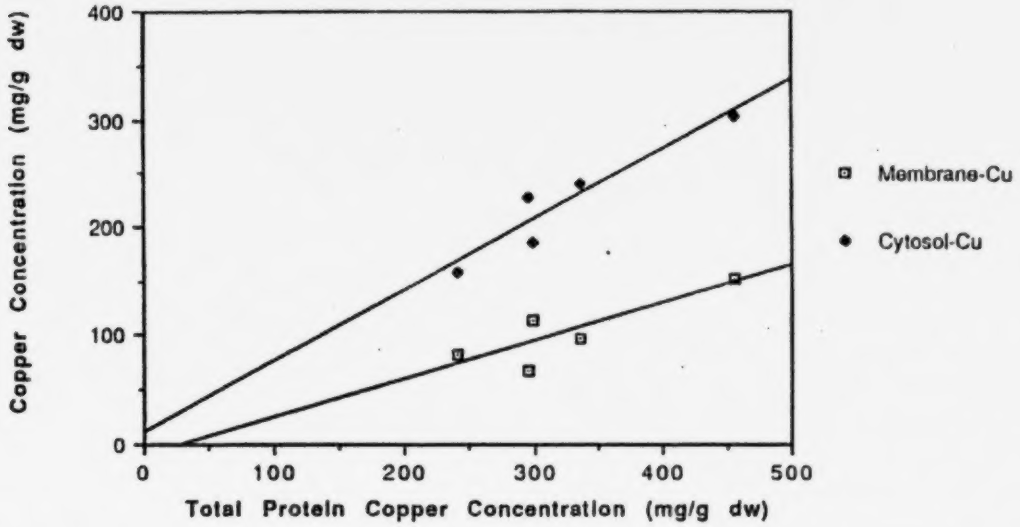


Figure 9: Hepatic Cytosol and Membrane Copper Concentrations as a Function of TPCC for Caged Rainbow Trout from the Tsolum River u/s Puntledge River

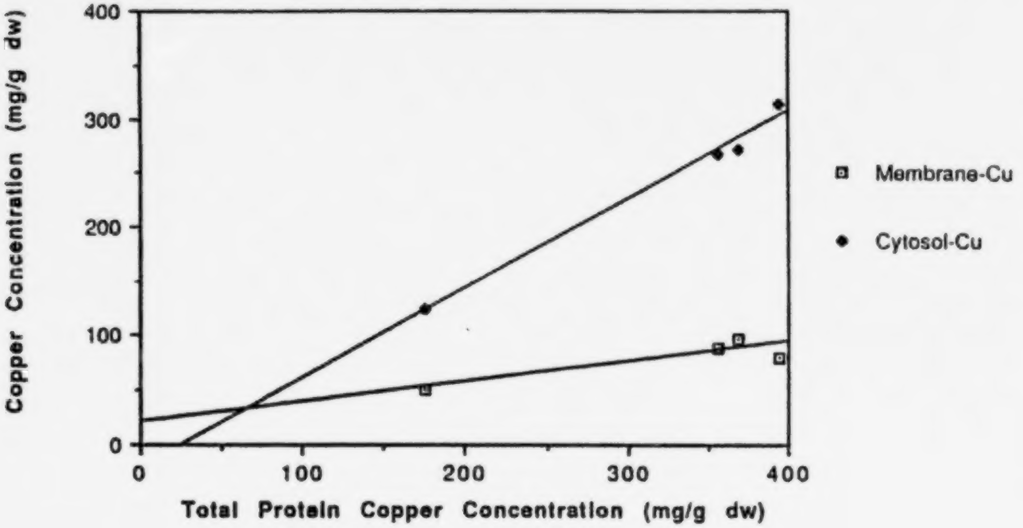


Figure 10: Hepatic Cytosol and Membrane Copper Concentrations as a Function of TPCC for Rainbow Trout from the Tsolum River near Farnham Road

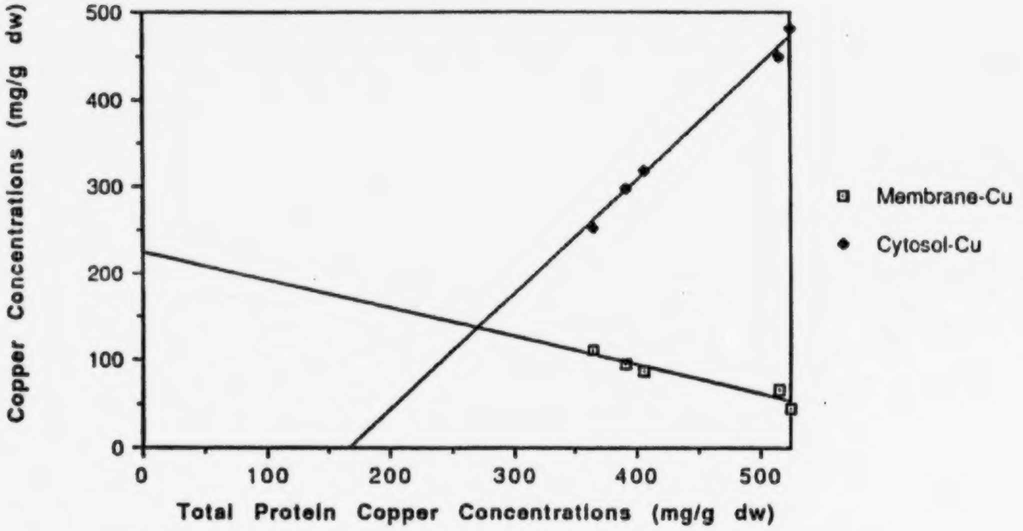


Figure 11: Cytosolic and Membrane Copper Concentrations vs Metallothionein Levels for Rainbow Trout in the Tsolum River u/s Puntledge River

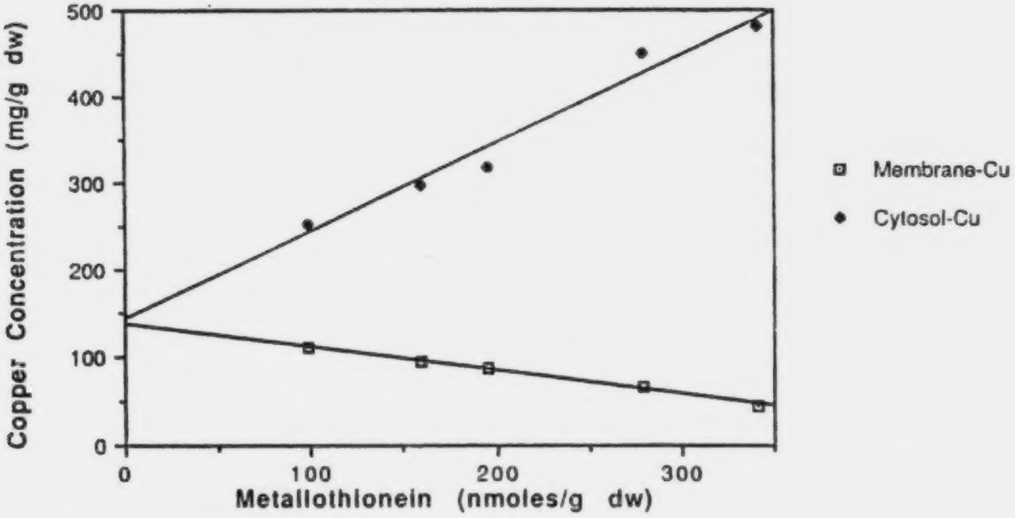


Table 15: Equations Between Cytosolic Protein Copper Concentrations and the Total Protein Copper Concentrations for Rainbow Trout

Site	T. Copper (µg/L)	Slope	Equation	r^2
All sites		0.56	$Y = 0.61 + 0.557X$	0.93
Maxwell Lake	L1	0.57	$Y = 0.11 + 0.574X$	0.90
Old Wolf Lake	L1	0.53	$Y = -0.05 + 0.525X$	0.97
Buttle Lake	2	0.65	$Y = 0.65 + 0.653X$	0.99
Stocking Lake	L1	0.74	$Y = 0.04 + 0.744X$	1.00
Tsolum:Duncan M.	3	0.65	$Y = 0.17 + 0.652X$	0.90
Tsolum:Puntledge	14	0.82	$Y = -0.33 + 0.822X$	0.99
Tsolum:Farnham R.	20	1.32	$Y = -3.51 + 1.324X$	0.99

The slope of the regression lines ranged from a low of 0.53 at Old Wolf Lake, to 1.32 at Farnham Road. A slope of 0.5 (cytosol vs TPCC) would indicate that the copper associated with the protein fraction was equally partitioned between the membrane and cytosol fractions. A slope of 1.0 would indicate that the proportion of membrane copper in the TPCC is constant, and any increase occurred in the cytosol fraction. A slope greater than 1 would indicate that the copper membrane concentrations decreased with increased TPCC levels, and cytosol fractions increased accordingly.

The slopes from Maxwell and Old Wolf lakes were between 0.5 and 0.6, indicating that in pristine lakes the partitioning of copper is similar between the cytosolic and membrane fractions. The high slope (1.32) on the Tsolum River upstream of the Farnham River indicated that the membrane copper concentrations decreased with increased TPCC concentrations (Figure 10). The inverse relationship between metallothionein and membrane copper (Figure 11), suggested that the decrease in membrane copper was due to the induction of metallothionein, the stripping of copper from the membrane fraction, and the accumulation of metallothionein copper in the cytosolic fraction.

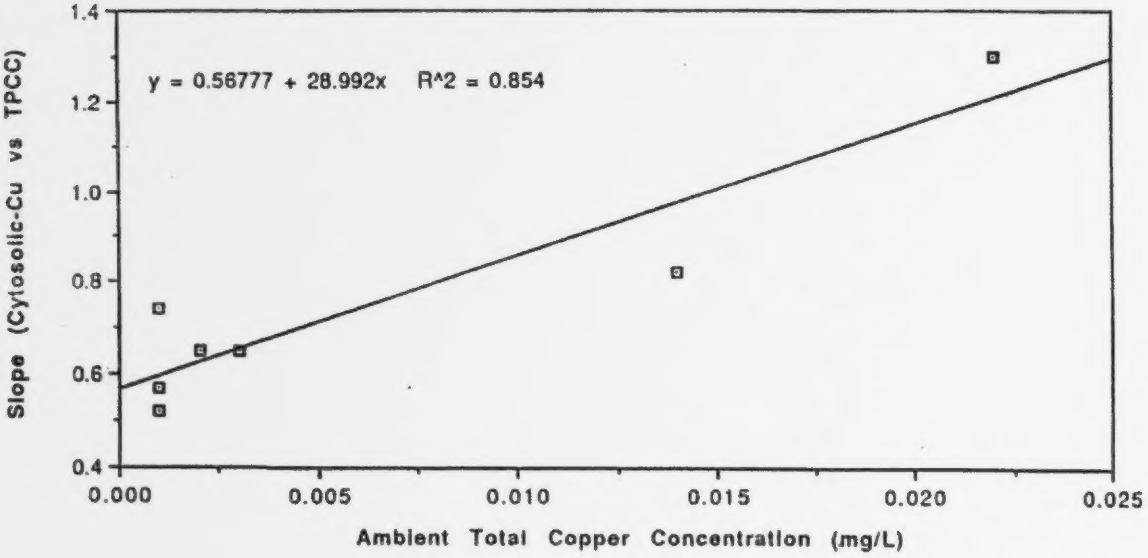
Statistical analysis of the slopes, showed significant differences at the 0.01 probability level between certain sites.

MAXWELL=OLD WOLF < BUTTLE=STOCKING=DUNCAN=PUNTLEDGE < FARNHAM

Buttle and Stocking lakes had a significantly greater slopes than Maxwell and Old Wolf Lakes. Puntledge was not significantly greater than Buttle, Stocking, or Duncan because of the small sample size, however, the slope at Farnham Road was significantly greater despite the small sample size and limited degrees of freedom.

The slope between cytosolic copper and TPCC had a high correlation with the ambient copper concentration (Figure 12). Although the correlation was highly significant ($r=0.925$: $P<<0.005$), there were two potential problems with the data. Firstly, the slope from Stocking Lake did not fit with the other data points, and secondly, the high slope at Farnham Road suggests that the relationship may not be linear. Despite these limitations, the high correlation warrants further investigation and refinement of the relationship.

Figure 12: Slope of Cytosolic Copper vs Total Protein Copper Concentration as a Function of Ambient Copper Concentration



DISCUSSION

The results from the Tsolum River watershed demonstrate that the 96 h LC_{50} bioassay is of little use in determining the effects of long term chronic toxicity. The 96 h LC_{50} is further limited in that it fails to distinquish toxic levels causing less than 50% deaths of fish. For the Tsolum River watershed, a 96 h LC_{50} using rainbow trout indicated acute toxicity at 49 µg/L. However, longer *in situ* exposure estimated a 21-day LC_{50} of 32±16 µg/L in 1989 and 16±10 µg/L in 1990. It is probable that longer exposure to this range of copper concentrations would result in increased mortality, or affect the reproduction and growth of the fish that survived. However, information from any bioassy is largely limited to the life stage used, as it is difficult to extrapolate toxicity information to earlier more sensitive life stages

In an attempt to quantify chronic responses in fish, total copper concentrations in the liver and muscle have been used. Unfortunately, total liver concentrations appear sensitive to relatively low levels of copper, yet insensitive to higher concentrations so that total liver copper concentrations do not correlate well with ambient levels. For example, total liver copper concentrations in rainbow trout from Buttle Lake are significantly greater than the control lakes, however concentrations have not changed from 1966 despite significant changes in ambient dissolved copper concentrations (Deniseger et al.,1990).

In contrast, over the same period of record, copper muscle levels in rainbow trout from Buttle Lake have decreased significantly in response to decreasing ambient concentrations. In fact, Buttle Lake rainbow trout copper muscle levels are no longer significantly different from the control lakes (Deniseger et al.,1990).

In a separate approach, Roch et al. (1982) were successful in correlating hepatic metallothionein concentrations in rainbow trout with the ambient copper (and zinc) concentrations in Buttle Lake. Based on laboratory experiments with Buttle Lake water and various copper concentrations (in association with zinc and cadmium) they proposed a metallothionein concentration of 500 nmoles/g (dw) as a safe level for salmonids. Roch and McCarter's proposed safe levels applies to the ratios of zinc, cadmium, and copper found in Buttle Lake, and correlated to concentrations of 0.05 mg Zn/L, 0.0025 mg Cu/L, and <0.0005 mg Cd/L (Roch and McCarter, 1984b).

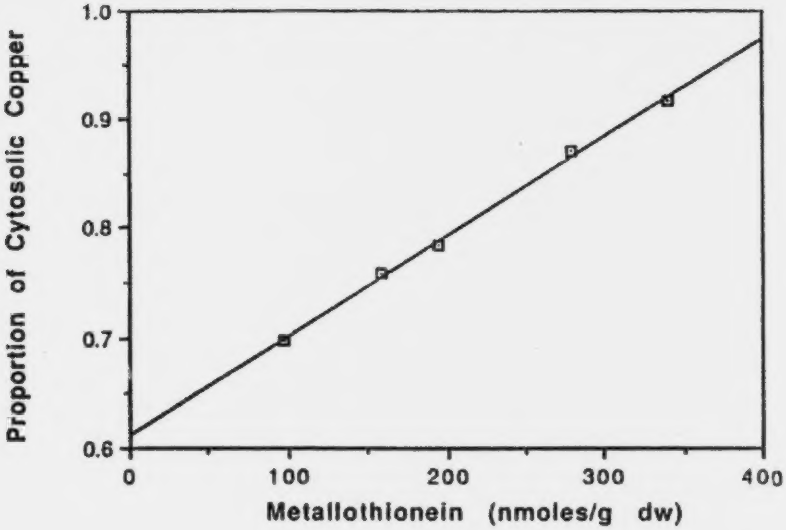
Although the caged rainbow trout in the Tsolum River demonstrated increased metallothionein concentrations with increased copper concentrations, their levels were not different than levels from sites with low copper conditions. Presumably increased exposure time would have resulted in increased metallothionein concentrations in the Tsolum River fish. Consequently, metallothionein measurements are limited to native populations, or transplanted fish that have been exposed for an extended (although undetermined) period of time.

The biochemical tests as proposed by Imber et al. (1987) complimented the work of Roch et al. (1982) through the partitioning of copper (or other metals) into either the cytosolic or the membrane fractions, and the copper concentration associated with the metallothionein like proteins. Biochemical analyses on rainbow trout livers from pristine lakes (e. g., Maxwell and Old Wolf Lake) had copper partitioned on an equal or 50:50 basis (cytosolic:membrane). With elevated copper exposure, the proportion of cytosolic copper increased and the membrane copper decreased.

The role of metallothionein in the shift between the cytosolic and membrane fractions was unclear in fish at low copper concentrations, but was highly significant in fish from the Tsolum River at Farnham Road and to a lesser extent in fish from the Puntledge site. At Farnham Road, metallothionein concentrations were strongly correlated with the cytosolic copper fraction and negatively correlated with the membrane copper fraction (Figure 11) The strong correlations implies a large metallothionein induction, and the removal of copper from the membrane (low copper affinity) to the metallothionein molecule (high copper affinity) in the cytosolic fraction. At Farnham Road, the proportion of cytosolic copper increased to approximately 90% of the TPCC in fish with the high metallothionein concentrations (Figure 13).

The slope of the relationship between cytosolic copper and TPCC was site specific, and increased with increased ambient copper concentrations. The result was a statistically significant relationship between the slope and ambient copper concentrations (Figure 12). This relationship allows the quantification of a response resulting from chronic exposure other than long term mortality. A method of measuring chronic responses, other than chronic mortality, is very important for monitoring the fisheries resources in watersheds like Buttle Lake and the Tsolum River that receive acid mine drainage for literally thousands of years.

Figure 13: Cytosolic Copper as a Proportion of the Total Protein Copper Concentration vs Metallothionein levels for Rainbow Trout from the Tsolum River at Farnham Road



Future research should focus on the linkage between the effects of chronic exposure on fish growth and reproduction, the length of time required for caged fish to respond to the metal levels, and the effects of other metals on the physiological response.

The British Columbia and CCREM criteria for copper in soft waters is 2 µg/L (Singleton, 1987; CCREM, 1987). The copper concentrations from Buttle Lake (2 µg/L) and the Tsolum River at Duncan Main (6 µg/L in 1989, and 3 µg/L in 1990) were at or slightly above the level of the criteria. Two coho and no steelhead mortalities were recorded in 1990 at Duncan Main, however, no coho mortalities were observed in 1989. It is unclear if the 2 coho mortalities at Duncan Main in 1990 were related to copper toxicity.

The biochemical data suggests that the rainbow trout at Duncan Main responded to the detectable copper concentrations, however, the low slope for the relationship between cytosolic copper and TPCC suggest that the chronic exposure is low. Based on the biochemical results at Duncan Main, rainbow trout in Buttle Lake are not expected to experience copper toxicity, which is in agreement with the 1989 metallothionein results and the metallothionein threshold (500 nmoles/g dw) recommended by Roch and McCarter (1984b).

Based on the results at Buttle Lake and Duncan Main, the B.C. criterion as recommended by Singleton (1987) appears to be adequate in terms of long term toxicity. Additional work on the chronic effects of copper on reproduction and growth must be completed for a thorough test the criterion.

Sampling Protocol: The principal purpose of a sampling protocol is to ensure that the data collected from different sites or projects will be comparable, allowing overview papers (like this) to pool the data and develop an understanding of the effects of metals on the fisheries resource. The proposed sampling protocol downstream of metal discharges requires both laboratory dose-response relationships, and *in situ* sampling downstream of the discharge.

Water Quality: It is important to sample for both total and dissolved metals, distilled water blanks to ensure there is no contamination of the samples, and general water chemistry in order to calculate the concentration of the Cu^{2+} ion.

Metals: Copper (0.001 mg/L detection level: ask for three significant figures e.g., 0.023 mg/L), special low level cadmium (0.0001 mg/L detection limit), lead (0.001 mg/L detection), and zinc (0.001 mg/L detection)
Cations: Calcium (diss.), magnesium (diss.), sodium (diss.).
Anions: Alkalinity (total and phenolphthalein = HCO_3^- and CO_3^{2-} estimates), sulphate (diss.), chloride (diss.).
Other parameters: pH, copper complexing capacity.

Fish Samples: Use native fish where available. Rainbow trout are preferred because of the ubiquitous nature of the species, and the existing data base. Other salmonids can be used, but their response to metal concentrations is yet to be determined. Ten fish per site is recommended for statistical purposes. In all cases record the length, weight, sex, and state of sexual development of the fish. Sexually mature fish should be avoided as the sex hormones may interfer with the induction of metallothionein.

Caged fish can be used in areas where the conditions have destroyed the native populations. The biochemical techniques (outlined below) should be used in conjunction with long term mortality measurement (e.g., Tsolum River). It appeared from the Tsolum data, that 28 days is not sufficient exposure time for metallothionein analysis, however, the induction of metallothionein was demonstrated and important in explaining the results at Farnham Road and upstream of the Puntledge River. Consequently, metallothionein data is useful, however it cannot be compared to results from native fish. It has not been established if 28 days is adequate time for the other parameters in the biochemical tests (e.g., cytosolic and membrane copper relationships) to become stable and comparable.

Caged fish are difficult to use because of the requirement to feed on a frequent basis, the stress induced transporting and caging the fish, and the importance of site selection of the cages so the fish are not in fast flowing water.

Laboratory experiments are useful in determining dose-response relationships for a given effluent (e.g., acid mine drainage). The major requirement is to ship large quantities of water to the laboratory doing the experiments. These type of dilution experiments are a useful in conjunction with native or caged fish data.

Biochemical Techniques:

Fish must be live caught and the extracted liver placed immediately in Tris Buffer and then on dry ice. Samples must be kept at >15°C prior to analysis. Parameters to be analyzed are:

Total Copper
Cytosolic Copper
Membrane Copper
Metallothionein
Metallothionein copper (optional)

CONCLUSIONS

There is a need to develop effective environmental monitoring programs downstream of industrial sites that have significant potential impacts on the aquatic environment. For chronic metal toxicity, the 96 h LC_{50} is inadequate. Based on the data presented in this publication, the existing water quality criteria (Singleton, 1987; CCREM, 1987) appears to be adequate in terms of chronic toxicity, however, in terms of the effects of copper on fish reproduction and growth (a measure of chronic stress), the existing criteria have yet to be tested.

The use of fish as biomonitors allows for the integration of water quality over a period of time. Native fish are prefered for metallothionein and biochemical analysis because we can assume they are inequilibrium with the ambient water quality conditions. Caged fish can determine chronic toxicity, as well as metallothionein and biochemical responses. It appears from the Tsolum River study that the metallothionein measurements from caged fish should not be compared to metallothionein measurements from native fish due to differences in exposure time. The measurement of metallothionein in caged fish experiments is encouraged becasue the induction of metallothionein (e.g., caged fish from the Tsolum River near Farnham Road) can corroborate the chronic mortality and biochemical data for that site.

The use of fish as biomonitors has the potential to be a more effective water quality monitoring program as it will integrate the water quality during events that may otherwise be missed by discrete sampling, and it has the potential for being less expensive. Future research should focus on the improved correlation of the biochemical responses and the ambient copper concentrations, and long term studies that link chronic stress of fish to changes in reproduction and growth.

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DIRECT AND INDIRECT MECHANISMS OF CHRONIC CONTAMINANT STRESS ON FISH POPULATIONS

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Chronic contaminant stress can be partitioned into direct and indirect components, both of which can be differentiated by the principal mechanisms or pathways by which they ultimately affect the structure and function of organisms. Direct effects of contaminants are usually expressed first at the cell or tissue level on components such as enzymes, metabolism, osmoregulation, etc. Effects on these lower-level functions are promulgated upward through increasing levels of biological organization and may be ultimately manifested as changes in growth and reproduction. Indirect effects of contaminant stress on organisms are expressed primarily through the food chain via the quality and quantity of energy available to fish. The MFO enzymes and DNA integrity can be used as indicators of direct exposure to contaminants while indicators of nutrition and metabolic energy pools serve as useful indicators of indirect contaminant effects on organisms.

SOME RESPIRATORY RESPONSES OF JUVENILE PACIFIC SALMON TO THE ANTISAPSTAIN CHEMICAL TCMTB. G.M. Kruzynski and I.K. Birtwell, Department of Fisheries and Oceans, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, B.C., Canada V7V 1N6 (604-666-7913).

EXTENDED ABSTRACT

The export value of British Columbia sawmill production is 4.1 billion dollars. Offshore markets, primarily to Japan, the UK and other EEC countries and Australia demand prime quality lumber; sales are valued at 2.1 billion dollars. However, prolonged transport and storage times required to reach these markets, result in conditions favourable to the growth of mould and fungi which may stain (sapstain) fresh lumber. To avoid this cosmetic problem, 90% of all lumber exported overseas is treated with biocides at a cost of approximately 18 million dollars. The benefits of antisapstain chemical use have been estimated at 388 million dollars (Deloitte et al. 1989).

Since the mid-1930's freshly cut lumber has been treated with the sodium salts of penta- and tetrachlorophenol. However, in British Columbia, environmental concern about the use of these biocides was increased in 1978 when high levels of residues were discovered in water, sediments and biota in the vicinity of sawmills.

Initially, it was thought that most of this contamination was due to poor handling practices at lumber treatment facilities, so a Code of Practice was formulated and recommended to the industry. However, by 1987, studies by Environment Canada documented that voluntary compliance was largely ineffective and several mills were charged under the federal Fisheries Act. Furthermore, studies demonstrated that high levels of chlorophenols could be leached from lumber stored outdoors awaiting shipment from export terminals. When the link between dioxins/furans and chlorophenols was established, several importing countries banned imports of chlorophenol-treated lumber.

In 1988, the lumber industry rapidly changed to alternative antisapstain chemicals. At the time, only three had been registered for use by Agriculture Canada, namely TCMTB [2-(thiocyanomethylthio) benzothiazole], copper-8 and borax. However, the extremely limited toxicological data base made available to the Department of Fisheries and Oceans rendered risk assessment to aquatic resources "unassessable" (Agriculture Canada et al. 1989).

By 1989, no investigations of potential sublethal effects on aquatic organisms had been reported, yet TCMTB comprised 50% of antisapstain chemical use in B.C. and 62% in eastern Canada. It was soon discovered that TCMTB could also be leached from lumber by rainfall, and concentrations of up to 400 ug·L^{-1} were reported in storm water discharging into the Fraser River (Krahn 1990). Serious concerns about potential impacts of such contamination on valuable salmon and other fishery resources stimulated the present research program.

Flow-through bioassays were conducted in 70 L annular exercise tanks with juvenile chinook and coho salmon in both fresh and sea water to establish lethal limits; 96 h LC50's fell into the range of 6-17 ug·L^{-1} TCMTB.

Behavioural observations indicating respiratory distress were followed-up by quantifying cough (gill purge) rate at various sub-lethal concentrations of TCMTB. Both the commercial formulation (Woodstat 30WB) and pure TCMTB were tested and found to elicit similar responses.

Breathing patterns were recorded from buccal-cannulated fish and dose-response relations determined. Each fish served as its own control.

A 6 h exposure to 10 $ug \cdot L^{-1}$ TCMTB increased cough rate from less than $1 \cdot min^{-1}$ to $20 \cdot min^{-1}$. Breathing rate did not change appreciably and it took several days for cough rate to return to baseline. On two occasions, recovery was interrupted by a secondary rise in cough rate in clean flowing sea water - suggesting a sensitization to other stimuli. The threshold (NOEC) was 4 $ug \cdot L^{-1}$ over a 6 h exposure period. Similar responses were observed over a longer (43 h) exposure to a low (1.8 $ug \cdot L^{-1}$) concentration of the toxicant.

Scanning electron micrographs demonstrated that gills of fish surviving a 96 h exposure to 10 $ug \cdot L^{-1}$ TCMTB were seriously damaged. The epithelium of the secondary respiratory lamellae appeared to be stripped. These results imply serious long-term damage and together with the dramatic disruption of the normal ventilatory pattern, suggest a compromised gas exchange.

Laboratory experiments were subsequently validated at a lumber mill discharging TCMTB-contaminated storm water into the Fraser River. Dye studies and chemical analyses of river water were used to delineate the lateral and vertical distribution of the mixing zone. A marked vertical stratification of the water column was used to advantage. Uncontaminated water pumped from a depth of 2 m provided the baseline respiratory pattern recordings. Contaminated surface water was then used to quantify cough rate in response to TCMTB present in the river.

Chinook salmon responded with a 6-7 fold increase in cough rate. The highest concentration of TCMTB detected during the experiment was 8 $ug \cdot L^{-1}$.

These results demonstrate the sensitivity and validate the utility of the cough rate response for detection of TCMTB in receiving waters.

ACKNOWLEDGMENTS

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The Toxicity of Chlorothalonil to Aquatic Fauna and the Impact of its Operational Use on a Pond Ecosystem

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Abstract. Chlorothalonil is a fungicide whose heavy use pattern in potato growing areas of eastern Canada gives it the potential for significant aquatic contamination. A study, which comprised a series of laboratory bioassays and several treatments of a pond system, was undertaken to determine the toxic effects of chlorothalonil on aquatic fauna. The bioassays consisted of acute exposures to rainbow trout (*Oncorhynchus mykiss*), water fleas (*Daphnia magna*), threespine stickleback (*Gasterosteus aculeatus*), blue mussels (*Mytilus edulis*) and soft-shell clams (*Mya arenaria*). Delayed mortality and reproductive disruption were also determined for *Daphnia*. Uptake and depuration by blue mussels at sub-lethal exposures were also determined. A subsequent field study determined the impacts of three separate operational sprays to a pond system through measurements of effects on caged aquatic fauna and impacts on endemic benthic invertebrates. The 96-h LC50 of technical chlorothalonil for rainbow trout was 76 μg/L and was not significantly different ($P < 0.05$) from that of the formulated product (Bravo^R 500). The 96-h LC50 of Bravo 500 for blue mussels and clams was 5.9 mg/L and 35.0 mg/L respectively while the 48-h LC50 of that product to *Daphnia* was between 130 μg/L and 200 μg/L. Chlorothalonil exposure of *Daphnia* to concentrations as low as 32 μg/L significantly ($P < 0.05$) increased the time to production of first young, but there were no delayed effects on survival, number of young produced or growth at concentrations of 180 μg/L or less. Chlorothalonil was initially accumulated by blue mussels to concentrations approximately ten times greater than exposure concentrations, however, tissue concentrations returned to the same level as exposure concentrations within 96 h. Spraying of ponds resulted in mortality of caged water boatmen (*Sigara alternata*) and threespine stickleback (*Gasterosteus aculeatus*) which could be related to chlorothalonil exposure, however, caddisfly larvae (*Limnephilus* sp.), freshwater clams (*Psidium* sp.), water beetles (*Haliplus* sp.), scud (*Gamarus* spp.) and midge larvae (Chironomidae) did not suffer substantial chlorothalonil-induced mortality. Likewise, changes in endemic benthic invertebrate abundance after sprays were not remarkable or consistently related to treatment. Faunal impacts in the pond were generally of a much smaller magnitude than were predicted by bioassay results. Factors such as dilution, adsorption to suspended particles and microbial degradation are thought to have attenuated the initial pond concentrations of chlorothalonil, thereby reducing their toxicity.

ENHANCEMENT TO THE PIELOU METHOD FOR ESTIMATING THE DIVERSITY OF AQUATIC COMMUNITIES

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ABSTRACT

The Pielou method of estimating the diversity of aquatic communities by plotting the relative abundances of the three most common species per sample on ternary plots, has been further developed. Algorithms are presented which permit the ternary plots to be readily produced via any computer spreadsheet having orthogonal graphics capability. Moreover it is shown that the Pielou method may be modified without loss of statistically significant information, so as to present time series diversity data as chronological tables or plots similar to those commonly used for other water quality variables, rather than as the ternary plots used by Pielou. Additionally the Pielou method has been extended from 3-dimensional to n-dimensional relationships. The usefulness of this new method for monitoring the impacts of toxicants to aquatic communities is demonstrated through examination of phytoplankton data for two oligotrophic lakes, one impacted by acid mine drainage and one pristine.

INTRODUCTION

Environmental scientists often describe and compare the diversity of biological assemblages or communities on a quantitative basis through use of various mathematical algorithms, and numerous papers have been published regarding species diversity indices and related algorithms including similarity indices, dissimilarity indices, richness indices, and evenness indices. Such indices are important to current ecological theory and are extensively used in environmental research; various papers offer comparisons between various indices and suggest criteria for selection (for example Lamont and Grant 1979; Wu 1982; Washington 1984; Sai and Mishra 1986;).

With regard to the aquatic environment, Pielou (1981) suggested that the great effort required to identify and count individual microscopic or taxonomically difficult organisms is prohibitively high with regard to the usefulness of the estimates achieved. Pielou recommends that greater effort be placed on collecting many samples from any water body being studied, with reduced effort being placed on the examination of each individual sample, and towards this end Pielou (1979, 1981) has developed a rapid method for estimating the diversity of each sample based upon the relative abundances of the three most abundant species within each sample. Pielou represented the species diversity of each sample as a single point on a ternary graph, and of each collection of samples as a swarm of such points. Visual inspection of the pattern taken by the swarm of "diversity points" on such a diversity plot yields a qualitative estimate of the diversity; all the points will fall within a right-angled triangle which Pielou called a "diversity triangle".

Where quantitative measure is required, Pielou recommended that the number of diversity points within each half area of the diversity triangle be counted and the ratio determined; where two communities need be compared, Pielou recommended that the number of points within each half area be counted, a 2 by 2 table set up and a χ^2 test performed. Two benefits of the Pielou method of estimating species diversity are that comparisons can be made between communities having no common species, and that historical records where only most abundant species were identified and counted can now be used on a quantitative basis.

Pielou solely exemplified her technique with communities of marine benthic foraminifera. This present paper shows that this technique is also useful with regard to freshwater phytoplankton communities. Additionally, this paper presents algorithms so that the necessary ternary graphs may conveniently be drawn as routine orthogonal X-Y graphs, and describes how the abundance ratio method can be extended to permit diversity results to be presented both chronologically and for ratios other than ternary.

METHODS

Statistical Calculations

Following the Pielou method, for every sample the three most abundant species were determined, each set sequenced into descending order, and the count for each of these three divided by the summed count for the three, yielding q_1, q_2 and q_3 such that $q_1 + q_2 + q_3 = 1$ and $q_1 \geq q_2 \geq q_3$. The author has determined that since the "diversity triangle" defined by Pielou is a 30-60 right triangle, then any single point defined by the three abundance ratios q1, q2 and q3 can in fact be represented precisely by a single pair of X-Y coordinates, where $X = (q_2 + q_3/2)/0.86603$ and $Y = q_3$. Therefore ternary graphs were readily produced via standard orthogonal graphics computer packages, for example via an EXCEL spreadsheet such as illustrated in Figure 1. The vertices of the diversity triangle are (0,0), (0.5773,0) and (0.5773, 0.3333).

Pielou suggested that the diversity of two species assemblages can be compared statistically (i.e. null hypothesis that the two diversities do not differ) by dividing each diversity triangle into two halves of equal area, counting the number of points within each area, preparing a 2x2 matrix and performing a routine chi-squared test. Examination of the trigonometry shows that the half-area dividing line intercepts the diversity triangle at (0.1691, 0.0976) and (0.5773, 0.0976), and moreover shows that the statistical test proposed by Pielou is dependent solely upon Y-values and is entirely independent of X-values. Since the X-values are little meaningful, the author recommends that abundance ratio based estimates of diversity be presented not as ternary plots but rather as chronological tables or chronological graphs. This permits chronological patterns to be visually obvious and presents the diversity data in a format similar to that commonly used for other water quality variables. For statistical tests identical to that described by Pielou, points may be counted above and below the geometric half-divider $Y = 0.0976$, a 2 by 2 table set up and a $\chi 2$ test performed as adjusted for continuity.

Pielou restricted her consideration of abundance ratios to the three most abundant ratios. However, this concept may be extended to the n most abundant ratios: since the n^{th} abundance ratio value always lies upon a line

Figure 1

A portion of the Buttle Lake phytoplankton data shown with numbers rounded for presentation. The three columns to the right of the Julian-like date are the relative counts (no./mL) for the three most abundant species in each sample; the column titles indicate the calculations performed. The rightmost two columns, X and Y can be used to draw points in their relative locations for a ternary plot; alternatively the date and Y columns may be used to create chronological plots.

BUTTLE LAKE PHYTOPLANKTON

DATE JULIAN YEARS	DATA				RATIOS				
	DOM. 1	DOM. 2	DOM. 3	SUM 1-3	*q1 = Dom1/Sum	*q2 = Dom2/Sum	*q3 = Dom3/Sum	X * = (q3/2+ q2)/0.866	Y * = q3
1966.62	14.70	4.50	3.80	23	0.639	0.196	0.165	0.321	0.165
1966.79	4.20	3.60	3.20	11	0.382	0.327	0.291	0.546	0.291
1967.62	28.80	15.90	9.60	54.3	0.530	0.293	0.177	0.440	0.177
1967.71	5.60	3.00	1.80	10.4	0.538	0.288	0.173	0.433	0.173
1980.63	26.20	12.20	6.40	44.8	0.585	0.272	0.143	0.397	0.143
1980.72	22.20	10.60	4.80	37.6	0.590	0.282	0.128	0.399	0.128
1980.80	25.80	7.00	6.60	39.4	0.655	0.178	0.168	0.302	0.168
1983.08	25.50	3.20	3.60	32.3	0.789	0.099	0.111	0.179	0.111
1983.23	110.40	6.80	2.40	119.6	0.923	0.057	0.020	0.077	0.020
1983.33	118.60	12.80	3.10	134.5	0.882	0.095	0.023	0.123	0.023
1983.42	731.00	85.70	6.20	822.9	0.888	0.104	0.008	0.125	0.008
1983.56	58.10	3.60	2.90	64.6	0.899	0.056	0.045	0.090	0.045
1983.65	19.00	13.50	10.90	43.4	0.438	0.311	0.251	0.504	0.251
1983.83	9.10	7.50	7.00	23.6	0.386	0.318	0.297	0.538	0.297
1984.13	53.80	31.70	5.30	90.8	0.593	0.349	0.058	0.437	0.058
1984.35	1157.00	206.50	38.50	1402	0.825	0.147	0.027	0.186	0.027
1984.48	2384.50	326.50	48.00	2759	0.864	0.118	0.017	0.147	0.017
1984.58	7334.50	326.50	19.00	7680	0.955	0.043	0.002	0.051	0.002
1984.65	2957.00	67.00	28.50	3052.5	0.969	0.022	0.009	0.031	0.009
1984.77	5385.50	31.00	10.00	5426.5	0.992	0.006	0.002	0.008	0.002
1984.96	9014.00	19.00	10.00	9043	0.997	0.002	0.001	0.003	0.001
1985.15	5061.00	28.70	19.10	5108.8	0.991	0.006	0.004	0.009	0.004

perpendicular to the base, then it will always be true that Y = q_n. The half-divider points as determined via Monte Carlo calculations. (single precision, 10000 iterations) yielded the following mid-array cut-offs for n = 2 to 5 respectively: 0.332, 0.153, 0.088 and 0.057; though these differ from geometric half-dividers (0.250 for n = 2; 0.0976 for n = 3), the situation is analogous to mean versus median, and results from the fact that all points within the diversity figures are not of equal probability. Statistical comparisons of identical data sets using each n and different half-dividers were performed to ascertain the relative impact each choice had on the power of both χ^2 and Student's t tests to distinguish between different data sets.

Phytoplankton Data

Phytoplankton data from Buttle Lake (site 0130082) and from Lizard Lake (site E206283) were examined. Both are oligotrophic lakes on Vancouver Island and both are surrounded by second growth timber. Buttle Lake is the largest in a chain of lakes in the Campbell River Basin, has its level controlled by the Strathcona Dam on the downstream Upper Campbell Lake, and is fed by several major creeks. Lizard Lake is located approximately ten miles from Port Renfrew, sits on a bench between Harris and Lens creeks about two miles from the San Juan River, and has no major inlets. Since 1966 Buttle Lake has received metals from a nearby mining operation, though this loading has been greatly reduced in recent years due to remedial measures; conversely Lizard Lake has little anthropogenic influence other than a small campsite.

Phytoplankton are sampled in Buttle Lake as a biological monitor of the potential impact of contaminants from the mine, and in Lizard Lake as part of a long term program watching for any acidification of lakes. Details as to sampling, preservation and analysis of the phytoplankton samples and also as to the water chemistry of Buttle Lake are described elsewhere (Deniseger et al. 1986, 1990).

RESULTS AND DISCUSSION

Ternary Graphs

Ternary graphs as described by Pielou were prepared for the Lizard Lake and Buttle Lake phytoplankton data (Figure 2). The two plots appear similar except Buttle Lake has more points in the left vertex, i.e. one species tends to dominate the abundance. However when third most abundant ratio values are plotted chronologically, as in Figure 3, it is very apparent that species diversity has behaved very differently in the two lakes in that for Buttle Lake loss of species diversity for extended time periods occurred spring and early summer 1983 and again from summer 1984 to spring of 1986. These time periods correspond to the persistent bloom of the diatom Rhizosolenia eriensis (Smith) described by Deniseger et al. (1986, 1990). Lizard Lake showed loss of diversity for short periods of time, not any long intervals.

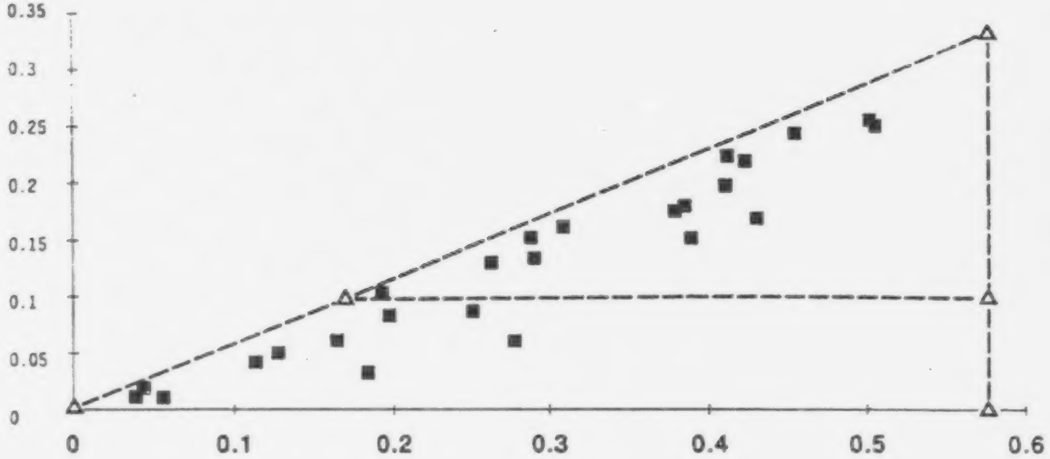
Similar patterns in species diversity for the two lakes are apparent when the n-most abundant ratio values are plotted chronologically, for $n = 2$ to 5, as presented in Figure 4. It may also be observed that as n increases, that the graphs flatten, obscuring differences between periods of high and of low diversity for the graph scale used. To the eye, It does appear, as Pielou had suggested, that one need not go beyond the three most abundant species and indeed one might well argue that $n = 2$ is quite adequate.

χ^2 tests, corrected for continuity, were unable at 95% confidence to distinguish any difference between the proportion of low to high diversity in phytoplankton data for the two lakes as estimated by the most abundant ratio method for $n = 4$ and $n = 5$, nor for $n = 2$ and $n = 3$ as estimated with the Monte Carlo half-dividers. However the χ^2 test did show a significant difference at 95% confidence but not 99% for both $n = 2$ and $n = 3$ as estimated with the geometric half-dividers.

Since the chronological plots for the Buttle Lake results show three periods of different diversity, namely higher diversity followed by lower diversity followed by higher diversity, with each of the periods about one-third of the number of samples collected, it seemed worthwhile statistically to compare each of these sub-records to Lizard Lake diversity. For the first of the three periods of record the χ^2 test showed no difference between the two lakes at 95% confidence for $n = 2,3,4,5$ and for both types of half-divider; however the χ^2 test could prudently be applied neither to either the second or third periods of data nor to the sum of the two since the minimum counts in the least proportions were not ≥ 5. Two-tailed F tests showed that there was no difference in variance at 95% confidence between Lizard Lake results and Buttle Lake results either

Figure 2

Pielou "diversity triangles" for Lizard Lake (top) and Buttle Lake (bottom) phytoplankton data. The data points are located in the relative positions they would have had on a ternary plot. The vertices of the diversity triangle are (0,0), (0.5773,0) and (0.5773, 0.3333); the half-area dividing line intercepts the diversity triangle at (0.1691, 0.0976) and (0.5773, 0.0976)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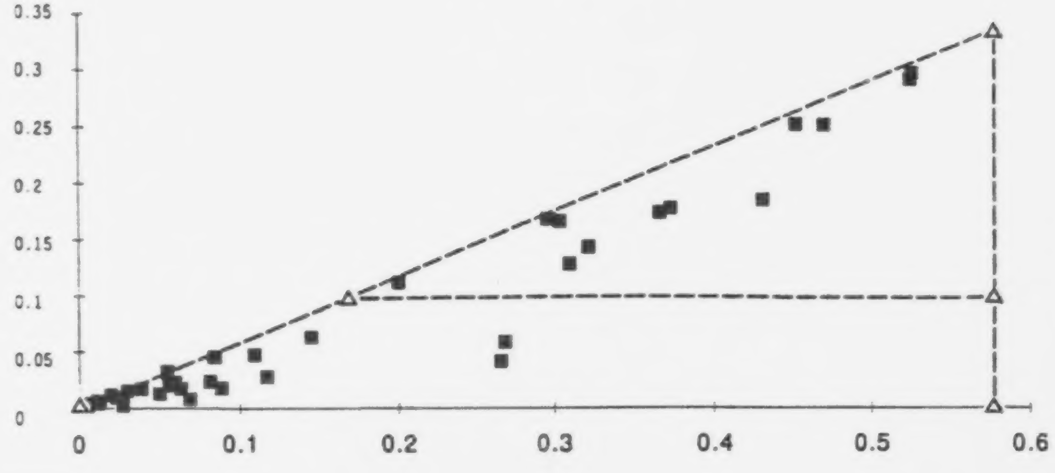
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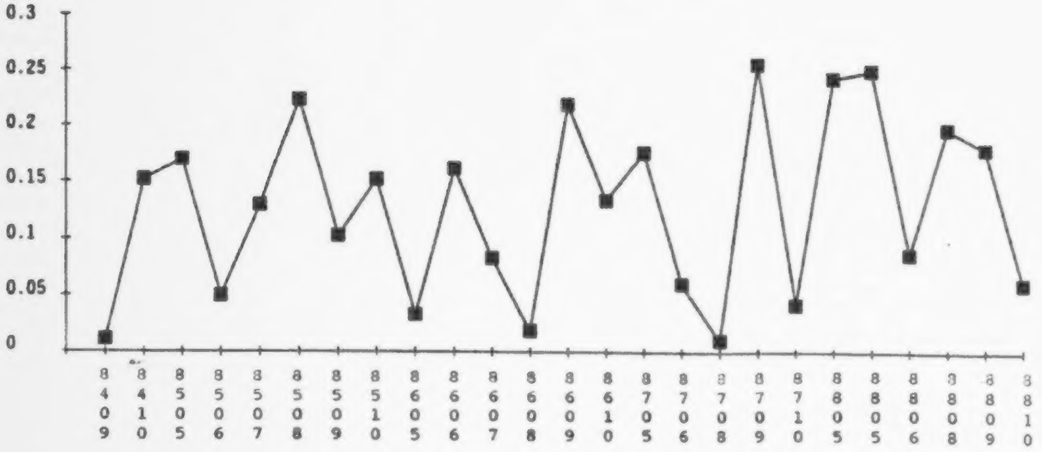
Figure 3

Chronological plot of species diversity for Lizard Lake (top) and Buttle Lake (bottom) phytoplankton data, as estimated from the abundance ratios of the three most abundant species,in each sample. For abscissa dates, 8409 is the 9th month of 1984. The Y-value for each point is identical to that it had when drawn on the Diversity Triangle, Figure 2.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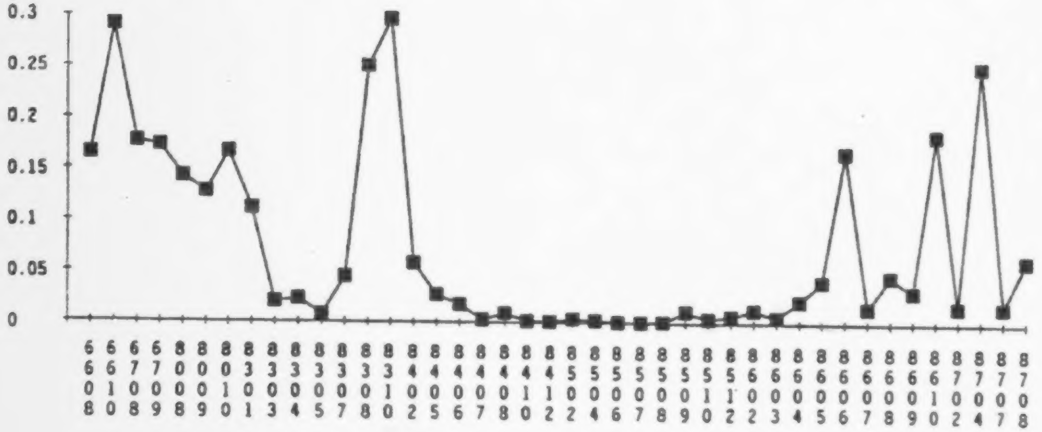
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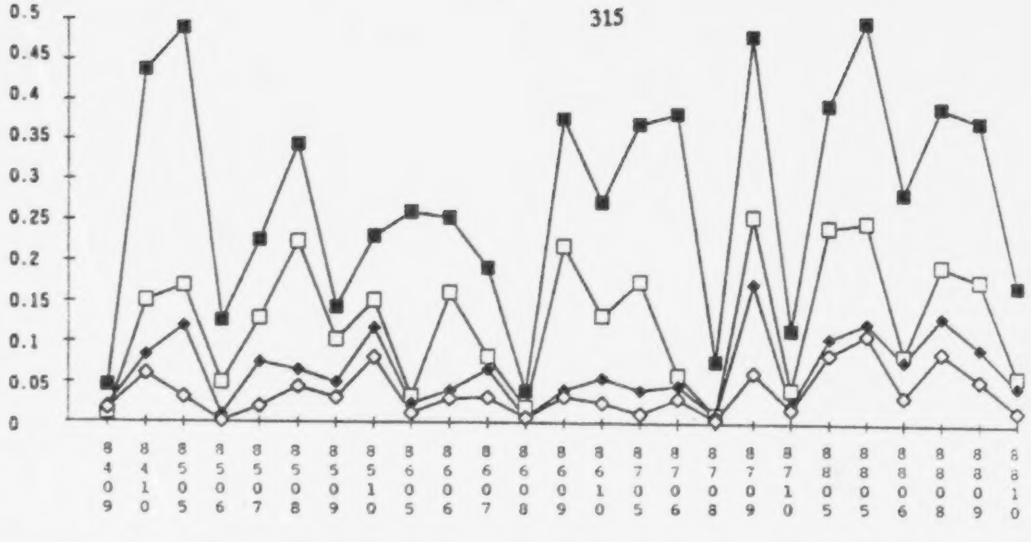
Figure 4

Chronological plot of species diversity for Lizard Lake (top) and Buttle Lake (bottom) phytoplankton data, as estimated from the two most abundant (open diamond), three most abundant (closed diamond), four most abundant (open square), and five most abundant (closed square) species in each sample. The Y-value for each point is the distance in (n-1)-dimensional space of the distance of the data point from the base of the "diversity figure".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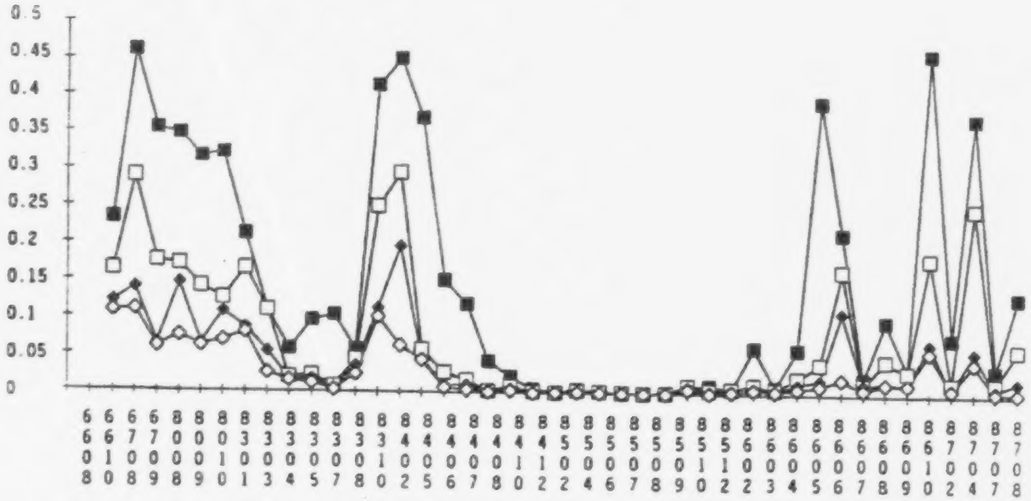
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in part or entire. Two-tailed Student's t tests failed to show any difference between mean diversity for Lizard Lake and for the first third of Buttle Lake data for n = 2,3,4,5 and also failed to show any significant difference between the Lizard Lake diversity and the entire set of Buttle Lake results for n = 5. However all other Student's t test showed Buttle Lake to be significantly less diverse than Lizard Lake at 95% and often 99% confidence. Thus it would appear that even after Buttle Lake had recovered from the extended period of low diversity, that its mean diversity was still reduced.

In summary, the Pielou method can be conveniently utilized via computer spreadsheet, can be modified so as to yield chronological graphs, and does appear to yield useful insights as to species diversity of freshwater lake phytoplankton. For this rapid method of estimating diversity there seems to be little benefit in examining more than the most abundant two species per sample, n = 2; also the Student's t test for statistical comparisons seems more in keeping than χ^2 test with the concept of a rapid test, and has the additional benefits both of avoiding the moot question of what type of half-divider to use, and of being able to handle data sets with very low counts in one of the proportions. The problem of whether the geometric or Monte Carlo half-divider should be used towards establishing proportions for the χ^2 test was not convincingly resolved but it is noteworthy that the χ^2 test and Student's t test gave consistent results where the geometric half-divider was used.

ACKNOWLEDGEMENTS

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USE OF FISH EMBRYO-LARVAL TESTS TO EVALUATE THE DEVELOPMENTAL TOXICITY OF RIVER WATER IMPACTED BY BLEACHED PULP MILL EFFLUENTS.

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Although embryonic development is the life-cycle stage most sensitive to the effects of toxicants, natural water sources have rarely been tested for embryotoxicity. We examined the effects of water from the Pigeon River, a stream which is severely impacted by bleached pulp mill effluents from a source in western North Carolina, on embryonic development in both the Japanese medaka, Oryzias latipes, a small killifish frequently employed in chemical toxicity and carcinogenicity testing, and the redbreast sunfish, Lepomis auritus, a centrarchid native to this stream system. Both medaka and sunfish embryos exhibited high incidences of lethal developmental abnormalities when raised in water collected downstream of the mill. In contrast, embryos of both species flourished in water collected either upstream of the mill or from the Little Pigeon River, a relatively pristine reference stream. Results of this and related studies suggest that acute embryotoxicity tests can successfully anticipate the longterm responses of fish populations to chronic contaminant exposure.

PLATFORM SESSION

Microscale Marine Toxicity Tests

Chair: P. Wells

Submitted to the 17th Aquatic Toxicity Workshop,
November 5-7, 1990 - Vancouver, B.C.

MICROSCALE MARINE TOXICITY TESTS - AN INTRODUCTION

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Microscale toxicity testing and experimentation, and the application of microscale "biotests" or "microbiotests" in the hazard assessment of effluents, chemicals and sediments has become an important approach in aquatic toxicology and marine ecotoxicology over the past two decades (for example, see Blaise 1990, Blaise et al. 1988, Burns et al. 1990, Long et al. 1990, Persoone and Wells 1987).

Early work in the marine field included pioneering studies by Jensen with phytoplankton, Woelke with oysters, Crisp with barnacles, Costlow and associates with crab larvae, Kobayashi with echinoids, and Rosenthal and Alderdice with fish embryos and larvae, to mention just a few (Jensen 1984, Wells 1984, Persoone et al. 1984). This has been followed by much recent work on a wide range of phyla and genera (from bacteria to algae, invertebrates, teleosts and amphibians), as shown by the session planned for this Workshop. Much research has taken place in the freshwater field, often stimulating or contributing to work on marine methods and marine species and life stages; work developing the freshwater and marine rotifer toxicity techniques is a classic example (Snell, pers comm., Snell and Vigerstad, this proceedings). Work and advancements in microbial ecotoxicology has contributed greatly to the wide range of exposure and analytical methods available for adoption or adaption to other "small species" or young life stages of macroscopic species (Blaise 1990, Dutka and Blaise, pers. comm.).

Many research and applied laboratories in North America and Europe are now incorporating various small-scale assays into hazard assessment schemes for water and sediments (Chapman, Swartz, Burns, pers. comm.). Sessions at recent SETAC meetings and many papers recently in Marine Environmental Research and Environmental Toxicology and Chemistry confirm this application. Methods are being discussed and standardized through the efforts of ASTM, and in Canada through the Intergovernmental Aquatic Toxicity Group of Environment Canada. In addition, microscale tests have a high potential for contributing to the broader objective of Status and Trends Marine Environmental Quality monitoring, using specific and non-specific biomarkers and bioindicators (see McIntyre and Pearce 1980, NRCC 1985, McCarthy and Shugart 1990, amongst others).

There are many advantages to assessing the acute and chronic, lethal and sublethal toxicities with small organisms, young life stages, and small systems, and possibly some important disadvantages. These are discussed below, in the individual session contributions. A premise of conducting microscale assays is that the advantages (availability of biota and test procedures, experimental control, statistical robustness, sensitivity, biological importance, cost, etc.) far outweigh the current disadvantages (ecological interpretation of effects, less tissue, higher level of required technical skills, etc.). It is hoped that the papers below contribute to a vigorous debate on this topic, and assist in identifying our current methodological capabilities and areas to focus research and applications.

Objectives of this session on Microscale Marine Toxicity Testing are:

1. To present the STATUS of current research methods with small-scale marine toxicity testing systems and organisms, covering as wide a range of organisms as possible;
2. To consider the COMPARATIVE SENSITIVITY of micro-scale testing methods, and the mechanisms of toxicity and other physiological/biochemical processes relating to the observed sensitivities;
3. To demonstrate APPLICATIONS of such approaches in the hazard assessments of marine contaminants, and in regulations, guidelines, and water quality objectives for pollution prevention and control;
4. To identify important NEEDS AND RESEARCH DIRECTIONS for the 1990's in microscale aquatic (marine) toxicity testing.

The session contributes to an overview of microscale marine toxicity testing, circa 1990, complementing the recent excellent synthesis of Blaise (1990). The session also raises awareness of the many exciting prospects and applications of this branch of aquatic toxicology in the continuing battle against water pollution.

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TECHNICAL EVALUATION OF THE SEA URCHIN FERTILIZATION TEST: PROCEEDINGS OF A WORKSHOP IN DARTMOUTH, NOVA SCOTIA

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ABSTRACT

Sea urchin fertilization tests have recently been developed by the U.S. EPA (1988) and ASTM (in draft form) for regulatory and investigative testing associated with the marine environments. The Dartmouth workshop was organized to investigate the suitability of the test for use by Canadian toxicity laboratories.

The test involves exposure of sea urchin sperm to an effluent or chemical sample and the endpoint is based on reduction of fertilized eggs relative to controls (expressed as EC50, NOEC, LOEC, chronic value).

At the workshop, two species of green sea urchin were tested: *Lytechinus pictus* (available year-round in spawning condition from the coast of California) and *Strongylocentrotus droebachiensis* (common to both Canadian coasts).

Limited investigation using a reference toxicant ($CdCl_2$) suggested comparable sensitivity between the two species. The test is simple, rapid, sensitive, and is similar in cost to other sublethal tests. Although additional method development is necessary, the test will likely be useful for toxicity testing related to Canadian marine environments.

INTRODUCTION

Traditionally, the standard acute toxicity testing with fish and invertebrates has been conducted to assess the toxicity of chemicals and industrial effluents to aquatic organisms. Recently, more sensitive short-term chronic assays have been developed, but their exposure time is usually over a period of four days and longer. There is still a need for a quick, sensitive and relatively simple assay to estimate the acute and sublethal effects of chemicals, commercial compounds and complex effluents. A number of Echinoderm protocols that have been developed by U.S. EPA, ASTM and others, and are used by a number of laboratories in North America. However, there is an immediate need for a standardized protocol to be used by Canadian aquatic toxicity laboratories.

In early May 1990, Environment Canada sponsored the Sea Urchin Fertilization Test Workshop in Dartmouth, Nova Scotia, to investigate the suitability of the test for use in Canada.

MATERIALS AND METHODS

Sea urchin (Echinoderm) fertilization bioassays were conducted according to the U.S. EPA Method 1008 (1988). Green sea urchin (*Strongylocentrotus droebachiensis*) common to all Canadian coasts and *Lytechinus pictus* from the coast of California were exposed to cadmium chloride ($CdCl_2$).

Sea urchins (*Lytechinus pictus*) were collected from California by Marinus Incorporated and shipped to BEAK laboratories by air express. Delivery was completed in 12 hours. Urchins were transferred to a static 135 L marine tank fitted with an under-gravel biological filter prepared with "Instant Ocean" sea salt which had been acclimated for three weeks previously. The tank was located in a 15°C temperature-controlled room. Ammonia, nitrite and pH were monitored routinely with a "Tetra" water testing kit. When the urchins were introduced, the salinity was 30 o/oo (SG 1.022 at 15 C). After 7 days, the sea urchins were transported to Environmental Protection Laboratory in Dartmouth, Nova Scotia. *Strongylocentrotus droebachiensis* were collected from the Atlantic coast by Darmouth Environmental Protection Laboratory personnel seven days prior to scheduled testing. They were kept at the EP Laboratory in Dartmouth at 9°C continuous-flow sea water (salinity 30 o/oo).

All urchins were held 24 hours prior to testing in the tank containing Bedford Basin sea water (salinity 30 o/oo), kept at 12°C, they were mobile and were actively feeding on romaine lettuce, both indicators of good health.

Toxicant concentrations were prepared with Bedford Basin sea water (salinity 30 o/oo) in concentrations of 3.12, 6.25, 12.5, 25, 50, 100 and 200 μg $CdCl_2$. Exposure concentrations were replicated in triplicate.

Thirteen male (mean weight of 9.5 g$\pm$4.3 and 27.8$\pm$3.8 cm outside diameter) and four female (mean weight of 15.6$\pm$5.2 g and 31.8$\pm$3.4 cm outside diameter) *Lytechinus pictus* urchins were selected randomly, injected with 0.5 mL of 0.5 M KCL and placed in individual petri dishes aboral side down in a half cm of sea water to collect sperm and eggs.

Concentrated sperm were composited from five males and a series of dilutions (25:1, 50:1, 100:1 and 200:1) of concentrated sperm stock were prepared. The sperm density of 200:1 dilution was counted in a haemacytometer and the required dilution of 25:1 stock was calculated to provide 5×10^7 sperm/mL. The final concentration of sperm in the test stock was verified with the haemacytometer.

Within 20 minutes of sperm collection, 0.1 mL of diluted sperm (7.3×10^7 sperm/mL) was added to 5 mL of each of the toxicant concentrations. After the sperm were exposed to the test solutions for one hour at which time the ova were introduced to determine sperm viability.

Eggs were also composited from female urchins providing about 50 mL of egg suspension. The stock was thoroughly mixed and the number of eggs in 0.1 mL was counted. The dilution required to contain 2,000 ova/mL was calculated and prepared.

After the one-hour sperm exposure, 1 mL of thoroughly mixed ova suspension was added to each of the exposure vessels. The ova suspension was remixed between test vessel additions to ensure an equal number of ova were introduced to each container. The ova were allowed to undergo fertilization for twenty minutes and at which time the developing fertilization membrane established.

The test was terminated by the addition of 2 mL of 10% buffered formalin.

The contents of each test vessel were then poured into an etched petri dish, and random squares were inspected and the number of fertilized and unfertilized ova were counted until a cumulative 100 were scored. Eggs from only two exposure replicates were scored, although the EPA method recommends at least three replicates.

The results were calculated according to Dunnett's procedure (Dunnett, 1955) to estimate no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values after arcsin transformation of data. The EC50 was estimated using the moving average (Stephan, 1977). The proportion of unfertilized ova observed in the controls was used to correct response data using Abbot's formula (Finney, 1971) in the EC50 calculation.

Results

Fertilization data for cadmium chloride are summarized in Table 1 and detailed data are presented in Tables 2a and 2b.

The fertilization EC50 was estimated using the moving average method after correction for control non-fertility using Abbot's formula (Finney, 1971). The fertilization response decreased

TABLE 1: CHRONIC RESULTS OF THE SEA URCHIN FERTILIZATION TESTS AFTER EXPOSURE TO CADMIUM CHLORIDE ($CdCl_2$)

Species	EC50* (μg/L)	NOEC (μg/L)	LOEC (μg/L)	Chronic Value (μg/L)
Lytechinus	33 (27-42)	12.5	25	17.7
Strongylocentrotus	36 (32-41)	12.5	25	17.7

EC50	-	concentration producing 50% inhibition of fertilization
NOEC	-	no observed effect concentration
LOEC	-	lowest observed effect concentration
Chronic Value	-	estimate of the threshold inhibition concentration (geometric mean of NOEC and LOEC)
( )	-	95% confidence limits

* Calculated according to the moving average method.

TABLE 2a: *LYTECHINUS PICTUS* FERTILIZATION TEST DATA CONDUCTED ON CADMIUM CHLORIDE ($CdCl_2$)

Concentration (µg/L)	Replicate	No. of Eggs Counted	No. of Eggs Unfertilized	Percent Unfertilized	Mean (x) Response	Mean Corrected Response* (%)
0	A	100	23	23		
	B	100	35	35	29	0
3.12	A	102	48	47	47	25
	B	105	49	47		
6.25	A	106	54	51	46	24
	B	100	42	42		
12.5	A	100	51	51	47	25
	B	105	45	43		
25	A	100	66	66	64	49
	B	101	63	62		
50	A	104	40	38	46	24
	B	103	55	53		
100	A	102	90	88	90	86
	B	109	100	91		
200	A	100	89	89	94	92
	B	100	99	99		

* Values corrected according to the Abbot's formula (Finney, 1971).

TABLE 2b: *STRONGYLOCENTROTUS DROEBACHIENSIS* FERTILIZATION TEST DATA CONDUCTED ON CADMIUM CHLORIDE ($CdCl_2$)

Concentration (µg/L)	Replicate	No. of Eggs Counted	No. of Eggs Unfertilized	Percent Unfertilized	Mean (x) Response	Mean Corrected Response* (%)
0	A	144	19	13	12	0
	B	146	14	10		
3.12	A	111	16	14	15	3.4
	B	126	19	15		
6.25	A	135	20	15	15	3.4
	B	133	18	14		
12.5	A	149	20	13	15	3.4
	B	139	22	16		
25	A	156	56	36	35	26.1
	B	157	53	34		
50	A	153	123	80	82	79.5
	B	156	131	84		
100	A	153	145	95	93	92.0
	B	165	150	91		
200	A	110	109	99	98	97.7
	B	122	118	97		

* Values corrected according to the Abbot's formula (Finney, 1971).

as the exposure concentration increased. Reproducibility among duplicates also appeared consistent (range of 0 to 15%).

The three lowest concentrations 3.12, 6.25 and 12.5 μg/L were statistically similar to control fertilization rates (for both tested sea urchin species). Fertilization was impaired at higher concentrations and virtually inhibited at the two highest 100 and 200 ug/L exposure level. The fertilization response was clearly concentration-dependent, and inhibition was virtually complete over the toxicant exposure range, suggesting that the toxicant effect on sperm was real.

When one replicate of the control for *Lytechinus pictus* was analyzed by the four workshop participants, a range of percent fertilization values of 55% to 85% was obtained. The use of different microscopes, and the lack of a standard criterion to score the egg as fertilized or unfertilized, were identified as the causes of this variability.

CONCLUSIONS

The Sea Urchin Fertilization Test Workshop was organized to evaluate the use of sea urchin gametes assay in toxicological testing. Sea urchin fertilization test was successfully conducted with two species of green sea urchins (*Lytechinus pictus* from California coast and *Strongylocentrotus droebachiensis* from Halifax area) and the endpoints (EC50, NOEC, LOEC and Ch.V.) of percentage of fertilized and unfertilized eggs were achieved with cadmium chloride.

Exposure time was only one hour and twenty minutes, but preparation for the test and scoring the eggs required about eight hours. Also, some additional time was required for results calculation and data interpretation.

It was determined that the test is simple, rapid (one day) sensitive and of similar cost to other chronic assays. However, there are further needs for standardization of test procedures through interlaboratory testing. Also, comparative sensitivity studies between other species and life stages should be determined. Commercial suppliers for recommended test species should be identified. Additionally, there is a general lack of data comparing relative sensitivities between sea urchins and other test organisms commonly used for toxicity testing (e.g., RBT, molluscs, stickleback, sanddollar).

Preparation and timing were found to be critical, as sperm remain viable for only a short period of time.

It was found that the shape of the fertilization membrane can vary. A standard criterion is required to destinguish between fertilized and unfertilized eggs. A series of photographs showing different fertilization membrane shapes would aid in standarizing scoring between different operators and laboratories for this test.

It was also recognized that the discussed assay should be supervised and conducted by experienced toxicity lab staff.

The Workshop Working Group recommended that, after a few modifications and standardization of existing sea urchin test procedures, the sperm bioassay may be used as a useful biomonitoring test to measure the toxicity of industrial effluents or chemicals discharged to the marine environment.

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MINIATURIZING A TOXICITY TEST BATTERY FOR SCREENING CONTAMINATED SEDIMENTS. P. E. Ross, L. C. Burnett, C. Kermode and M. Timme, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820-6970, U.S.A. (217-244-5054).

INTRODUCTION

Environmental samples from areas of suspected pollution may contain complex mixtures of contaminants. In such cases, it is widely accepted that one cannot rely on a single bioassay with a 'most sensitive species' to detect all potential hazards (Cairns 1984). Different types of organisms respond to different classes of contaminants, so batteries of tests including species from different levels of biological organization are now generally used (Ross and Henebry 1989). An ideal suite of bioassays should maximize the yield of non-redundant information. If two tests show the same response pattern for most contaminants, there is a high degree of redundancy and only one of them should be included in the battery. Conversely, a pair of tests that have complementary sensitivities would tend to produce more non-redundant information.

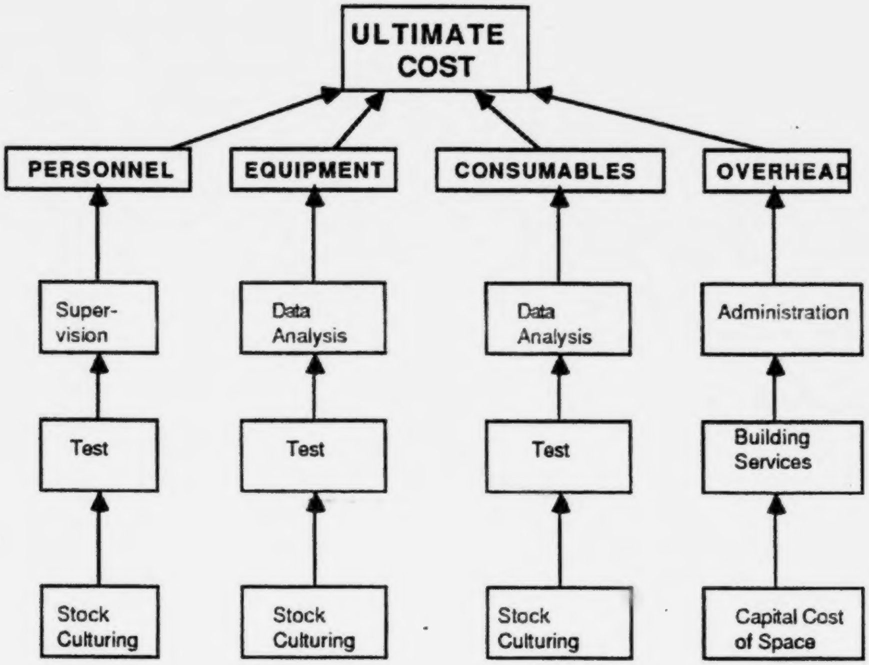
Contamination screening survey designs often call for large numbers of sampling sites, in order to ensure adequate coverage and sufficient resolution for mapping procedures. In these situations, a cost-efficient battery of tests is required. In this study we examined a broad spectrum of existing bioassay methods in order to select a suite of bioassays meeting our individual criteria (sensitivity, rapidity, replicability, cost and sample volume) and, as a group, providing a broad range of biotypes and an acceptable degree of non-redundant information.

METHODS

An initial selection was based on literature information on individual test criteria. From this phase, a short list of candidate tests was subjected to more intensive scrutiny. Sensitivity was assessed by parallel tests with 10 reference toxicants (4 metals, 6 organics). Rapidity was determined as the minimum time between sample receipt and the availability of results. Replicability was assessed by calculating coefficients of variation for replicate tests with reference toxicants.

Cost was determined by applying a complex assessment of all resources required to perform a bioassay (Figure 1), but commercial profit was not included. Sample volume is often a consideration for storage and transportation, and is particularly critical in applications where expensively and laboriously produced extracts are the final test article. Efforts were made to miniaturize candidate tests as much as possible to reduce sample volume.

Figure 1. Schematic of detailed cost calculation for bioassays.


ULTIMATE COST
PERSONNEL
EQUIPMENT
CONSUMABLES
OVERHEAD
Super-vision
Data Analysis
Data Analysis
Administration
Test
Test
Test
Building Services
Stock Culturing
Stock Culturing
Stock Culturing
Capital Cost of Space

RESULTS AND DISCUSSION

The resulting group of bioassays includes four tests: the Microtox™ *(Photobacterium phosphoreum)* bacterial luminescence assay (Bulich et al. 1981); an algal photosynthesis test *(Selenastrum capricornutum)* based on 14-carbon uptake (Ross et al. 1988); a lettuce *(Latuca sativa)* root elongation test (Wang 1987); and a rotifer *(Brachionus calyciflorus)* survival assay (Snell and Persoone 1989).

The recommended battery encompasses two basic life-form divisions (prokaryotes and eukaryotes) and three kingdoms (Monera, Plantae, Animalia). Both a vascular plant (lettuce) and a lower plant (the alga) are included. The algal cells are easily cultured and the organisms for the other three tests can be stored in suspended stages for long periods with no maintenance required. The longest exposure time is 96 hours (lettuce) and the other three tests all take 24 hours or less. Space requirements are minimal and a total of 7 hours of technical time is needed for the setup and breakdown of the four tests. The bioassays are all highly repeatable, with coefficients of variation ranging from 10.5 to 18.0 (Table 1). The entire battery can be run for a total cost of US$250, and only 36 ml of sample are required, including replication and a full dilution series (Table 1).

Table 1. Summary of evaluation criteria for four miniaturized bioassays. (C.V. = coefficient of variation)

Organism	Measurement	Source of Organism	Exposure/ Technical Time	Sample Volume	Reps	C.V.	Cost
Selenastrum capricornutum	^{14}C uptake *via* freshwater green alga	laboratory culture	24 /2 hr.	5 ml	5	18.0	$120
Microtox®	luminescence in marine bacteria	freeze-dried culture available commercially	15 min/1 hr.	3 ml	2	15.6	$ 40
Brachionus calyciflorus	survival of freshwater rotifer	cultured from cysts	24 /1 hr.	5 ml	4	12.0	$ 40
lettuce	root elongation of lettuce seeds	commercially available seeds	96 /3 hr.	23 ml	4	10.5	$ 50
TOTALS			**96/7 hr.**	**36 ml**			**$250**

Taken as a group, the four tests exhibit a great deal of sensitivity. For each of the reference toxicants used in the study, at least one test in the battery is at or beyond the sensitivity level of the water flea *Daphnia magna* and the fathead minnow *Pimephales promelas*, two of the most commonly used freshwater test systems. The suite of tests also satisfies the group criterion for non-redundant information. Each bioassay ranked as having the most sensitive response to at least one of the ten reference toxicants (Table 2). This indicates that the four tests are complementary, each having its own role in enhancing the overall sensitivity of the battery.

It must be remembered that screening-level tests measuring acute toxicity, such as those described above, cannot replace more costly bioassays using locally relevant species and measuring chronic responses and full or partial life-cycles. Nevertheless, the four bioassays in the recommended battery do measure endpoints and processes that are common to all aquatic ecosystems: electron transport, photosynthesis, growth and survivability. Screening tests should be viewed as operationally defined detection systems, used to identify problem areas where more resources and intensive effort should be focused. There is an inevitable tradeoff between direct environmental relevance and the measurement of chronic effects, on the one hand, and the capacity to evaluate large numbers of samples at a reasonable cost, on the other hand.

We have used this battery extensively in field assessments of sediment contamination. Our typical strategy is to score responses to each bioassay on a semi-quantitative scale, and then sum the toxicity scores for all four assays for each sample tested. The composite scores can then be mapped on a sampling grid to give a readily understandable picture of hazard distribution in the study area.

Table 2. Ranking of EC50 values of four toxicity test systems for ten reference toxicants. A value of 1 indicates the most sensitive response, while 4 indicates the least sensitive.

	Microtox	Algae	Lettuce	Rotifer
Cadmium	4	1	3	2
Copper	3	1	4	2
Nickel	4	1	3	2
Zinc	3	2	4	1
Σ ranks (inorganics)	**14**	**5**	**14**	**7**
Benzene	3	4	2	1
Methoxychlor	3	4	2	1
Pentachlorophenol	3	4	1	2
Phenanthrene	2	1	4	3
Phenol	1	3	4	2
Toluene	1	4	2	3
Σ ranks (organics)	**13**	**20**	**15**	**12**
Σ ranks (all chemicals)	**27**	**25**	**29**	**19**

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BATTERY OF TESTS APPROACH TO SEDIMENT HAZARD ASSESSMENT. B.J. Dutka, A. Jurkovic, R. McInnis, K.K. Kwan, and D.L. Liu. Rivers Research Branch, Department of Environment, NWRI, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6 (416) 336-4923.

The seemingly simple testing of river sediments to ascertain their toxicant/genotoxicant load is a task fraught with pitfalls. Three of the major problems which arise after the sediments have been collected are: the bioavailablity of the toxicants/genotoxicants, the representativeness of the sample and the extraction/concentration procedure to be used. The procedures followed in our laboratory to ameliorate some of these concerns will be presented along with a description of tests which are part of our battery of tests approach; i.e., Microtox, Mutatox, SOS-Chromotest, Toxi-Chromotest, ATP-TOX System, Algal Tox, *Daphnia magna*, *Ceriodaphnia dubia*, *Spirillum volutans*, Nematode, ECHA dipstick, spot plate, seed germination and root elongation and earthworm. Data will be presented illustrating the observations that the ability to elicit positive toxic/genotoxic responses, in the various bioassays, was influenced by the concentration/extraction procedures used on the sediments.

SUITABILITY OF MULINIA LATERALIS AS A EURYHALINE TOXICITY TEST SPECIES

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ABSTRACT

The mactrid clam, Mulinia lateralis, is being investigated as a representative marine/estuarine bivalve species for the U.S. EPA's Complex Effluent Toxicity Test Program (CETTP). The proposed test procedure is a modification of the 48-hr oyster embryo test. Laboratory cultures of adult M. lateralis have been maintained in spawning condition year-round. Successful embryological/larval development has been obtained at salinities ranging from 7 to 35 ppt. Precision tests using $CuSO_4$ and sodium dodecyl sulfate (SDS), conducted at 10 and 30 ppt salinities, had mean coefficients of variation of approximately 25%. The sensitivity of the M. lateralis embryo test to one organic and five inorganic compounds was comparable to, or greater than, published values for two commercially important bivalves, Mercenaria mercenaria and Crassostrea virginica. Because of it's precision, sensitivity, and ease of conduct, the M. lateralis test appears to be a suitable test method for inclusion in the marine test series.

EXTENDED SUMMARY

The 1972 United States Clean Water Act mandated the United States Environmental Protection Agency (EPA) and state agencies to implement a strategy of best available technology (BAT) and numerical criteria to achieve water quality control of toxic pollutants discharged to the Nation's waters. In 1984 amendments to this act modified the National Pollutant Discharge Elimination System (NPDES) to allow the inclusion of effluent toxicity testing requirements in the permit. In response to this, EPA sponsored the Complex Effluent Toxicity Testing Program (CETTP) which has developed a series of short-term toxicity tests designed to determine lethal and sub-lethal effects of effluents on freshwater (USEPA, 1989) and marine and estuarine organisms (USEPA, 1988).

One organism being considered for inclusion in the CETTP suite of saltwater tests is the coot clam, *Mulinia lateralis*. This small (< 2 cm shell length) mactrid bivalve ranges along the Atlantic coast from Malapeque Bay, Canada to Florida and in the Gulf of Mexico to northeastern Mexico (Rhodes, et al, 1972). It is primarily found in fine sediments (occasionally in surf-stirred sand) (Smith, 1964) and has been reported at salinities ranging from 1.4 to 75.1 ppt (Breuer, 1957). Because of its small size, laboratory populations of adults can be held in small (five gallon) aquaria and maintained in spawning condition year-round. Brood stock can be obtained from either field collections or laboratory reared larvae.

The toxicity test currently under development is adapted from existing bivalve embryo/larval tests (ASTM, 1989; Standard Methods, 1975). Basically, the test procedure involves spawning 10 to 15 animals by thermal stimulation and allowing fertilization to occur in the spawning dishes. Developing embryos less than two hours old are added to scintillation vials at a concentration of 75 embryos/ml. The vials contain 10 ml of test water and are maintained at room temperature (21°C) for 48 hours. The larvae are then microscopically examined and the number of normal larvae (i.e., those having a shell) determined. Statistical analyses of the test results are based on the reduction in percent of normal larval development. Point estimates, such as EC50, are calculated using Probit Analysis (Finney, 1971). Hypothesis testing using Dunnett's Procedure (Dunnett, 1955) may be used to estimate NOEC and LOEC values.

A series of experiments were performed with embryos obtained from adults held at 10, 20 and 30 ppt. Tests were conducted to determine the salinity range and precision of the *Mulinia* procedure. Additional tests were also conducted to determine the sensitivity of *Mulinia* to several heavy metals and one organic compound. These results were compared to published values for *Mercenaria mercenaria* and *Crassostrea virginica*.

Successful larval development was obtained at salinities ranging from 7 to 32 ppt. The upper salinity limit was not determined. Optimum control development was obtained when the tests were conducted within ± 5 ppt of the adult's holding/spawning salinity. Exposures of embryos to five concentrations of sodium dodecyl sulfate (SDS) and copper sulfate ($CuSO_4$), each at 10 and 30 ppt salinity, resulted in copper EC50 values of 17.7 and 17.1 µ/l, respectively. The SDS EC50 values were 8.2 and 5.8 mg/l, respectively. The corresponding coefficients of variation were 19.7% (Cu, 10 ppt), 4.7% (Cu, 30 ppt), 18.3% (SDS, 10 ppt), and 55.2% (SDS, 30 ppt). The additional test exposure of *Mulinia* embryos to copper, cadmium, nickel, lead, zinc, and phenol resulted in EC50 values equal to or lower than values reported for *Mercenaria mercenaria* (Calabrese and Nelson, 1974) and *Crassostrea virginica* embryos (Calabrese et al, 1973).

The Mulinia lateralis embryo/larval development test is easy to perform, has comparable precision to other CETTP toxicity tests (Morrison, et al, 1989), and, unlike most other bivalve species, can be conducted at any time of the year. Based on these observations and on comparisons with two commercially important bivalve species, the Mulinia lateralis procedure appears to be a reliable and sensitive toxicity test for marine or estuarine applications.

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USING DOSE-RESPONSE DATA FROM THE ROTIFER ACUTE TOXICITY TEST FOR BRACKISH AND MARINE ENVIRONMENTS

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INTRODUCTION

The purpose of this talk is to discuss the evaluation of short-term marine toxicity assays using the acute toxicity assay for the rotifer Brachionus plicatilis as an example. The goal is to provide a perspective that can be used to most effectively evaluate the rotifer model and other short-term biological models of toxicity.

THE MODEL

The model of toxicity is the brackish water species Brachionus plicatilis (Snell and Persoone, 1989; Snell et al., 1990). The cysts of this species and a freshwater congener, Brachionus calyciflorus are commercially available. Within 24 hours cysts stored on the laboratory shelf can be hatched and made available for the evaluation of toxicity.

The basic materials needed to complete an acute assay are simple: cysts, a 24 well plate, a transfer pipet, a fluorescent lamp, a dissecting microscope, and some wet chemistry capability. The acute assay is conducted over 24 hours. Methods are described in Snell et al., 1990 and Bio-Response Systems, 1990.

Some of the advantages of using the cysts are a reduction in the cost of culturing organisms, reduction in some of the factors known to influence quantitative variability, and standardization of species and strain between laboratories.

EVALUATION OF "SENSITIVITY"

The search for "sensitivity" of a model can be divided into two categories which we have termed absolute and relative toxicity. The definition of absolute toxicity is the potential risk a substance poses to the viability of closely related species. If one has concluded that rotifers are an important component of the ecosystem in which one is working, then the question for evaluation of sensitivity under absolute toxicity is:

> Does this rotifer model adequately represent all rotifers?

Under absolute toxicity, types of considerations in the evaluation of sensitivity include geographic distribution, habitat, physiological ecology and quantitative dose-response. Traditionally, for purposes of quantitative comparison, investigators have relied on the LC50 as an indicator of "sensitivity." In our example, Brachionus is a euryhaline species found in littoral zones on six continents. Table 1 shows some representative LC50 results for metals and organic compounds. In general, Brachionus appears to be more sensitive to metals than to organics.

We propose three potential definitions for relative toxicity. The first is:

> Differences in biological reactivity which are evidence of a variance from an accepted standard.

Under this definition one is interested in whether or not the model can show a measurable response that is predictive of a response known to be important (a standard). Standards often used are expected responses of fish, regulatory criteria, engineering or operational criteria, or public health criteria. This type of evaluation should make use of the dose-response characteristics of the model. For example, the LC50 for selenium is 17 mg/l. If you had a standard of 8 mg/l you might conclude the rotifer assay is not sensitive enough. However, the LC20 for Brachionus is 5 mg/l. Using 100 animals, it should be quite easy to use Brachionus to detect an LC20. Thus Brachionus is sensitive enough.

A second definition for relative toxicity is:

> Biological reactivity in response to changing environments.

To evaluate the usefulness of this model under this definition, the dose-response curve is again important. A measure of the usefulness of those curves is the coefficient of variation (C.V.) of the slopes. Three to four measurements were made for each of the four compounds used as examples in this talk. The C.V.'s for the slopes of the four compounds ranged from 5.8 to 24.1%.

Imagine an environment contaminated with 0.08 mg/l of each compound. Which compound will take the greatest effort to clean-up so that a reduction in toxicity is seen? Figure 1 shows the LC50's of mercury and silver. A 50% reduction would bring silver to below its threshold of toxicity of 0.05 mg/l. A similar

TABLE 1 - LC50 DATA FOR THE MARINE ROTIFER BRACHIONUS PLICATILIS. VALUES ARE IN MG/L.

	LC50		LC50
Mercury	0.06	Selenium	17
Copper	0.06	Nickel	>20
Silver	0.1	Ammonia	38
Tributyl tin	0.3	Cadmium	39
Lead	>4	Acetone (1 hr)	75
Zinc	>4.8	Chromate	102
NaOCL (1 hr)	1	Phenol	>400
NaPCP	2	2,4-D	598
CDNB	2	Diesel Fuel	345 ul/L
Chloroform(1 hr)	2.5		
Sodium lauryl sulphate	6		

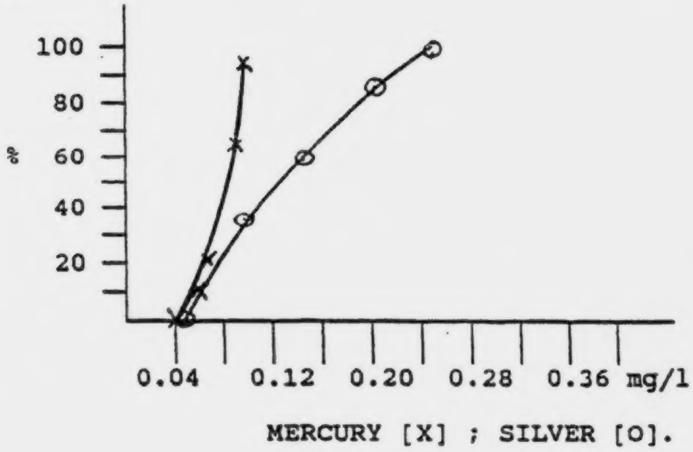

Figure 1. Percent mortality versus concentration of mercury and silver in an acute assay of Brachionus plicatilis. Mortality is the average of four assays.

reduction of mercury would also bring it to below the detectable level of 0.04 mg/l.

The LC50 for acetone is 75 mg/l. The assay may not be sensitive to acetone, compared to other aquatic invertebrates. But between 72 mg/l and 78 mg/l the slope of concentration versus % mortality is 13.5 (Figure 2). Because a 20% increase in rotifer mortality can be detected, a 1% increase in acetone concentration can be detected within that range, which makes this rotifer a sensitive indicator for some situations.

A third definition of relative toxicity is toxicity relative to other species. Under this definition of toxicity there are various considerations that may be important. For example, one may be interested in a model of a particular type of tissue, organ or cellular level response. Rotifers have catecholaminergic neuronal structures, a fixed number of nuclei (eutely), and a syncytium. Might dose-response curves of rotifers exposed to compounds known to be toxic to cell membranes, for example, prove useful for understanding mechanisms of toxicity?

As a second example, one might be interested in predicting the effects of a single pollutant in different environments. For selenium the LC50 for Brachionus plicatilis, the marine species, and Brachionus calyciflorus, the freshwater species, is the same (16 - 17 mg/l). Does this imply they are equally sensitive? The dose-response curves for the two species indicate a difference in toxicity (Figure 3). For plicatilis, the curve suggests little, if any threshold of response in the marine environment. For calyciflorus, there is a threshold at around 12 mg/l. So plicatilis is the more "sensitive." However, the slope calculated from % mortality vs concentration for calyciflorus is around 4.3. The slope for plicatilis is approximately 2. So, calyciflorus might be able to detect a change in concentration in the range of 12 to 30 mg/l more readily than plicatilis. Which is the more "sensitive?" What are the reasons for the differences? Is it biological or chemical? Consider an environment contaminated with 25 mg/l selenium. Do these curves suggest the cleanup would be less demanding in the freshwater environment than the marine?

DISCUSSION

Statements about the sensitivity of proposed models should include the type of toxicity one is evaluating. A complete evaluation should include knowledge of the dose-response curve. Data that leads to progress in accurate evaluation should include: no-observed-effect-concentration (NOEC), range of linearity, and slope.

There will not be an unlimited number of animal models that will

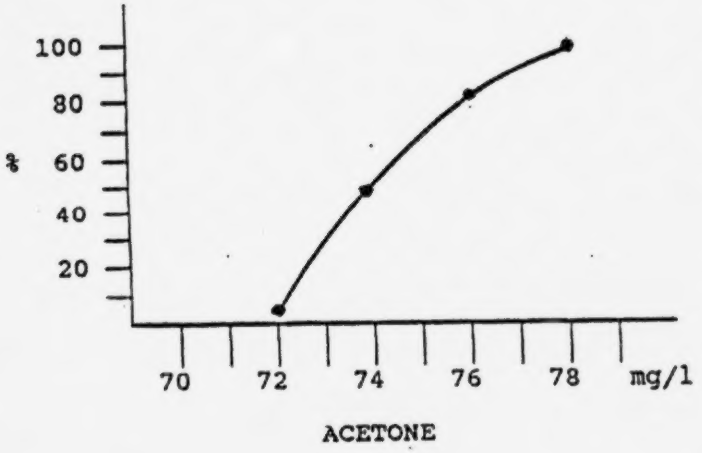

Figure 2. Percent mortality versus concentration of acetone in an acute assay of *Brachionus plicatilis*. Mortality is the mean of three assays.

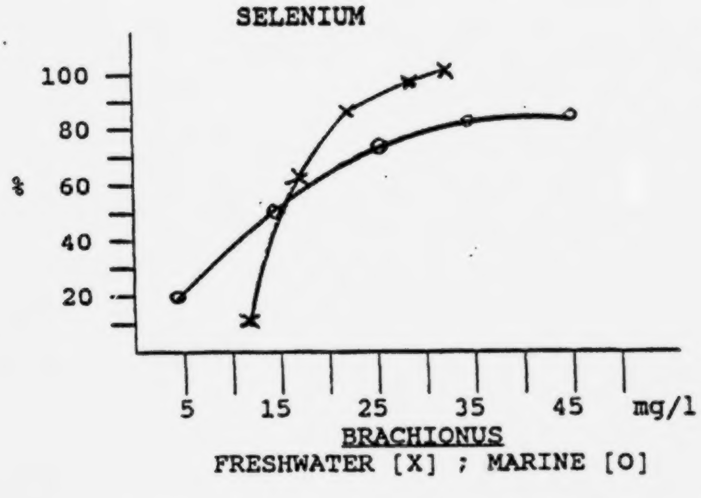

Figure 3. Percent mortality versus concentration of selenium in an acute assay of *Brachionus plicatilis* (marine) and *Brachionus calyciflorous* (freshwater). Mortality is the mean of 4 assays for each species.

be inexpensive, easy to maintain, and easy to accomplish. They are expensive to develop and the overall market is small. Environmental budgets are small. Unqualified statements such as "this model is not sensitive enough" can hinder progress in the development and use of biological models.

CONCLUSION

The real question for the evaluators of short-term marine assays is:

> Does the model respond in way that allows a useful prediction?

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RELATING TOXICITY AND BIOASSAYS. M.R. Samoiloff Bioquest International, #3-2725 Pembina Highway, Winnipeg, Manitoba, Canada (204)-269-7264.

This presentation addresses the issue of false positives and false negatives. In a study with 143 sediment samples, 23 were found to contain one or more priority chemicals at concentrations greater than acceptable limits. Only 13 of these showed toxicity with a battery of biological toxicity tests. Of the 110 samples that would be considered no-risk on the basis of chemistry, 54 showed significant toxicity to biological assays. In a study with 236 water samples, 119 had unacceptable levels of priority contaminants, and 97 showed toxicity with bioassays. Of the 117 water samples with no or low concentrations of priority pollutants, 65 showed toxic effects in bioassays. In using batteries of bioassays, different biological systems show strikingly different sensitivities and responses, with no test system consistently outperforming other test systems over a range of contaminated environmental samples. Approaches to comparing results of batteries of bioassays will be discussed.

CONTINUOUS-FLOW TOXICITY TESTS USING THE MICROSCOPIC LIFE STAGES OF VARIOUS MARINE ORGANISMS

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ABSTRACT

Ideally, aquatic toxicity testing should involve a variety of phylogenetically different organisms, their most sensitive life stages, and systems that allow close control of both toxicant dosing and water quality. Therefore, we have both developed a unique continuous-flow testing system and modified previously-developed early life stage tests for use in testing the comparative toxic effects of volatile organic chemicals and complex mixtures. The system is based on a simple exposure design utilizing premixed chemical stocks, may be entirely closed to the surrounding atmosphere to reduce chemical volatilization, incorporates exposure chambers designed for use with microscopic life stages, and, to prevent surface partitioning, is constructed entirely of inert materials (Teflon®, glass, and silicone). It has been successfully used with the sensitive early life stages of topsmelt (*Atherinops affinis*), mysids (*Holmesimysis costata*), red abalones (*Haliotis rufescens*), and giant kelp (*Macrocystis pyrifera*) to investigate the comparative toxic effects of a surfactant-based oil spill dispersant, Corexit 9527®. Due to the unique design of the system, it may accomodate either constant or declining toxicant water concentrations (to more accurately model environmental impacts). The combination of our unique system with early life stage tests allows the most sensitive evaluation of the environmental impacts of either volatile toxicants or complex mixtures, both of which may be difficult to manage in the laboratory.

INTRODUCTION

Continuous-flow exposure systems are desirable for most aquatic testing applications (APHA, 1985). Flow-through systems alleviate problems encountered in static tests, including oxygen depletion and excretia accumulation, and allow for improved water quality control (Mount and Warner, 1965; Mount and Brungs, 1967; Benoit and Puglisi, 1973; Garton, 1980; Benoit *et al.*, 1982; Hong *et al.*, 1987). Usually, continuous-flow tests are performed with systems designed to provide either serial (Warner, 1964) or proportional (Benoit *et al.*, 1982) dilution, and have been used successfully in testing with later life stages (juvenile or older) where organism containment is not a serious problem. However, due to their increased sensitivity, tests utilizing microscopic life stages are becoming more common. A system utilizing submerged fine-mesh baskets has been previously described for flow-through testing with mollusc larvae (Roberts, 1980); however, for containment tests with microscopic organisms have usually been static (APHA, 1985).

Most variations to the basic diluter design employ some degree of gravity-dependant water movement from one mixing container to another through tubes which empty into aquaria. Since the problem of volatilization has rarely been addressed, some or all of the mixing containers and aquaria are open to the atmosphere. However, many toxicants tested are complex mixtures in which the various components all have different physical-chemical properties, including water solubilities, volatilities, and Henry's law constants. Test results may be seriously effected by uncontrolled changes to their makeup. This is particularly important in petroleum-related toxicity research.

While open systems may be acceptable for inorganic and single organic toxicants where water concentrations are closely monitored, they are less appropriate for testing complex, volatile organic mixtures. The system described here was designed for oil spill dispersants, which generally are

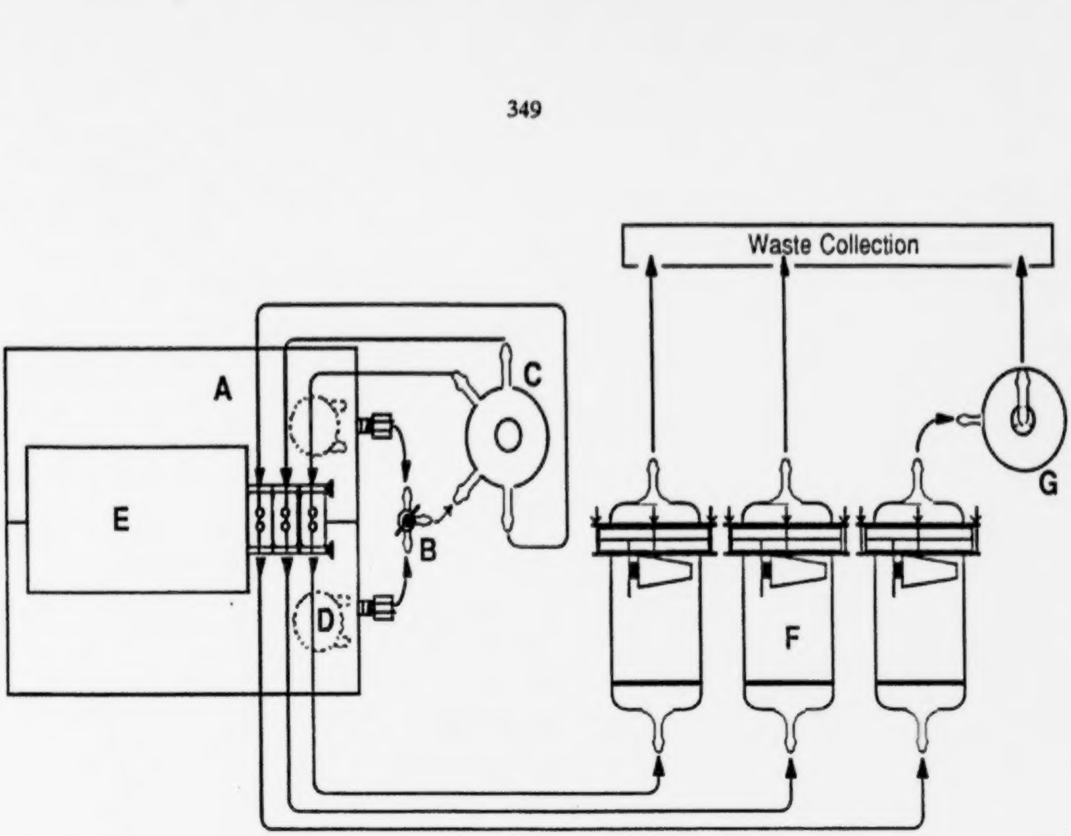

Figure 1. Overhead view showing the water flow pattern of each exposure cell. A, Teflon® stock reservoir bags; B, three-way stopcock; C, delivery manifold (aeration optional); D, water-driven magnetic stirrer; E, peristaltic delivery pump; F, replicate exposure chambers; and G, water quality sampling flask (adapted from Singer *et al.*, 1990a).

complex mixtures of surfactants and solvents; however, it has wide application in aquatic toxicity testing (Singer *et al.*, 1990a). Closed systems are now widely accepted and necessary for the testing of either petroleum (usually the water-soluble fraction) or petroleum-dispersant mixtures (NRC, 1989).

Certain features of the system design were incorporated to overcome limitations imposed by surfactant chemistry. However, they do not limit its usefulness in testing other complex organic mixtures. Our system does not involve automatic dilution; oil spill dispersants tend to block containment frits over time, seriously altering dilution patterns. Instead, stock solutions of each toxicant concentration are mixed daily and individually introduced into the system. Each stock concentration is delivered to replicate test chambers directly via peristaltic pump to provide highly accurate flow rates of as little as 0.1-0.2 L h^{-1}. The capability of using low flows is advantageous when testing the earliest life stages of many aquatic species, which may have little or no motility. Also, all components consist of Teflon®, glass, or silicone to minimize chemical partitioning.

This system has been used extensively to conduct tests with both metals ($ZnSO_4$ or $CuSO_4$) and the oil spill dispersant Corexit 9527®. Tests have been conducted with microscopic life stages of four marine species: giant kelp (*Macrocystis pyrifera*), red abalones (*Haliotus rufescens*), kelp forest mysids (*Holmesimysis costata*), and topsmelt (*Atherinops affinis*; Singer *et al.*, 1990a,b,c).

SYSTEM DESIGN

The system consists of six independent cells, representing five toxicant concentrations and a control. In each cell stock solution flows by gravity from the stock reservoirs (A), through a three-way stopcock (B), to a delivery manifold (with optional aeration; C), which is mixed by a magnetic stirrer (D; Fig. 1). The aerated sample is then pumped (E) to three replicate exposure chambers

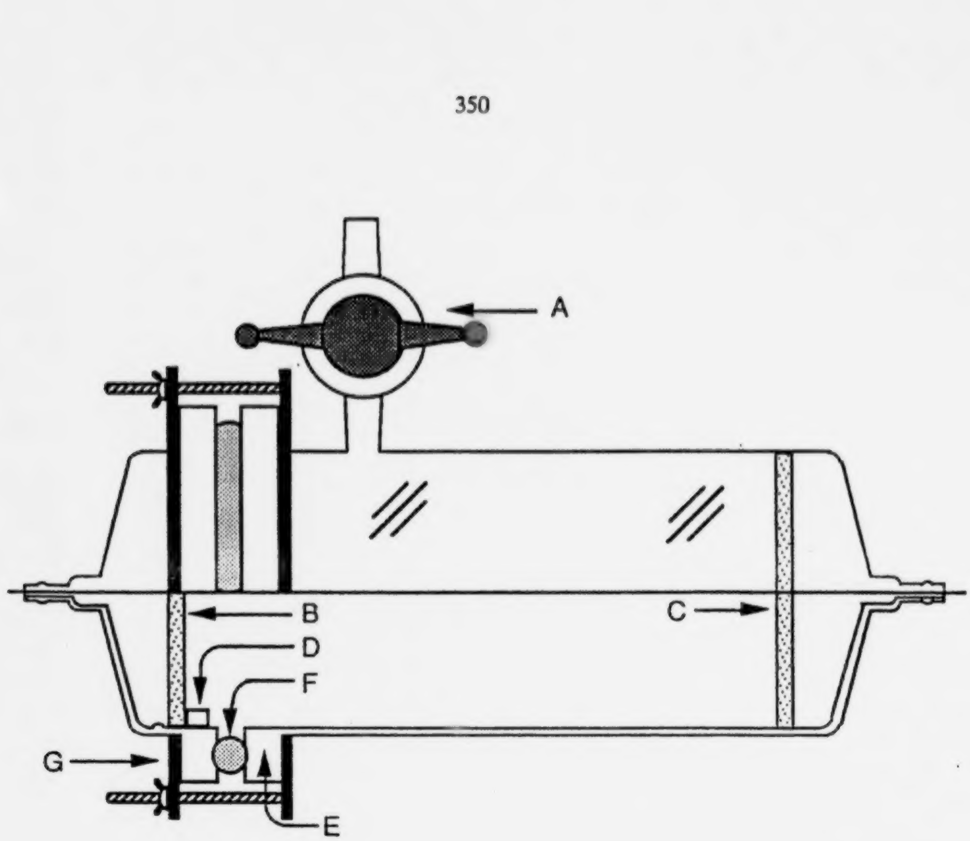

Figure 2. The flow-through exposure chamber. A, stopcock; B, removable fritted disk (40-60 µ); C, permanent fritted disk (40-60 µ); D, Teflon® frit-retaining ring; E, glass flange; F, silicone O-ring; and G, stainless steel U-clamp (adapted from Singer *et al.*, 1990a).

(F). A sampling flask is placed downstream of one chamber for water quality testing (G). By introducing aeration into the manifolds, oxygen levels can be easily maintained without significant chemical degradation of the stock solution, which may result from direct aeration of the stock reservoirs. When testing highly volatile chemicals, aeration is not appropriate and the flask may be sealed. In testing oil spill dispersants, however, aeration is needed to mitigate loss of dissolved oxygen due to surfactant action.

Stock solutions are mixed in Teflon® gas sampling bags (5 L, 4 mil; AeroVironment, Monrovia, CA), each equipped with a Teflon® bulkhead coupling, to minimize loss due to transfer between multiple containers. The bags are flexible, air-tight, and collapse upon emptying without need for an open headspace. Thus, they assure minimal stock degradation during testing from volatilization or surface partitioning. To maintain uniform mixing, each bag is equipped with a Teflon®-coated stir bar and placed atop a water-driven magnetic stirrer.

The delivery manifold is a 125 mL erlenmeyer flask fitted with a ^{24}/40 ground glass joint and four sidearms (3.1 mm i.d.); one sidearm acts as an inlet while the others supply the three replicate exposure chambers via the delivery pumps. Optional aeration is provided by an aquarium air pump and Pasteur pipet. When aeration is not required, the headspace is evacuated and the manifold sealed with a Teflon® sleeve and ground-glass stopper. The Teflon® bag is elevated and placed at a slight angle to insure hydrostatic equilibration with the aeration flask while the bag is emptied. Also, manifold aeration avoids direct chamber aeration, which may physically injure sensitive organisms

Water delivery is controlled with Masterflex® Model 7523-00 digital unified-drive peristaltic pumps (Cole-Parmer, Chicago, IL) equipped with Masterflex® Model 7016-21 lexan-bodied pump heads and stainless steel rollers. Utilizing Masterflex® silicone tubing (3.1 mm i.d., size 16), the

Table 1. Water quality measurements obtained with the exposure system in 48- and 96-h toxicity tests. Data shown are overall measurement ranges (from Singer *et al.*, 1990a).

Toxicant	Test organism	Temperature (°C)	DO_2 (mg L^{-1})	pH
Metals	Mysids	12.8 - 13.4	6.61 - 8.78	7.87 - 7.95
	Red abalones	15.2 - 15.3	7.54 - 7.89	7.88 - 8.00
	Giant kelp	12.8 - 12.8	7.39 - 7.69	7.86 - 8.03
Corexit 9527®	Mysids	14.9 - 15.6	6.18 - 7.69	7.75 - 7.94
	"	11.2 - 12.0	6.11 - 8.01	7.64 - 7.96
	"	14.6 - 15.2	6.01 - 7.35	7.60 - 7.87
	Red abalones	14.5 - 14.6	7.16 - 8.03	7.78 - 7.86
	"	13.5 - 13.6	6.95 - 7.77	7.75 - 7.95
	"	14.8 - 15.8	6.64 - 7.47	7.78 - 8.01
	Giant kelp	12.0 - 12.1	7.10 - 7.60	7.78 - 7.89
	"	12.7 - 13.2	7.56 - 8.34	7.76 - 8.03
	"	12.8 - 13.1	6.93 - 7.82	7.69 - 7.95

pumps can accurately deliver flow at rates as low as 1 mL min^{-1}; high water flows may injure non-motile microscopic organisms.

The aquatic exposure chamber was designed to provide: (1) containment of microscopic organisms without causing physical injury; (2) continuous low-flow to maintain acceptable water quality and minimize toxicant loss due to degradation (physical, chemical, or biological) or partitioning; and (3) atmospheric isolation to prevent uncontrolled toxicant loss from volatilization (Fig. 2). It is a glass cylinder, with a Teflon® and glass stopcock mounted on top (A), and sintered glass fritted disks (40-60 μ mesh) at each end (B,C). The stopcock allows introduction of organisms or food without permitting atmospheric exposure, while the frits provide containment for most larval organisms. Each chamber has one removable (B) and one permanently mounted frit (C), with the removable one held by a Teflon® retaining ring (D). The chamber design allows for the maximum inter-frit distance within a minimum overall chamber length, thus maximizing organism containment volume (165 mL). A glass flange (E) fitted with a silicone O-ring (F) and stainless steel U-clamp (G) seal the chamber. The two-piece design allows chamber access for collection of organisms, glass settling slides, other contents, and cleaning.

Sampling flasks (125 mL erlenmeyer flasks) for monitoring water quality are placed downstream of the third replicate exposure chamber of each concentration. Each is fitted with a $^{19}/_{22}$ ground glass joint, which accomodates an Orion Model 97-08 dissolved oxygen electrode (Cambridge, MA), and allows access for specific ion or pH electrodes. Also, each flask has an inlet port near the bottom and an outlet port at the top of a ground-glass stopper, also sealed with a Teflon® sleeve.

Manifolds, chambers, and sampling flasks are all placed in a rectangular water bath constructed from marine-grade plywood. Temperature control is provided by a refrigerated water circulator, and a pedestal pump is used for bath circulation and to drive the magnetic stirrers under each stock reservoir bag.

SYSTEM EVALUATION

Utilizing our system, toxicity tests involving the microscopic early life stages of giant kelp, red abalones, mysids, and topsmelt with metals or Corexit 9527® have been conducted (Singer *et al.*, 1990a,b,c). Generally, testing has required two reservoir bags per concentration, connected by a

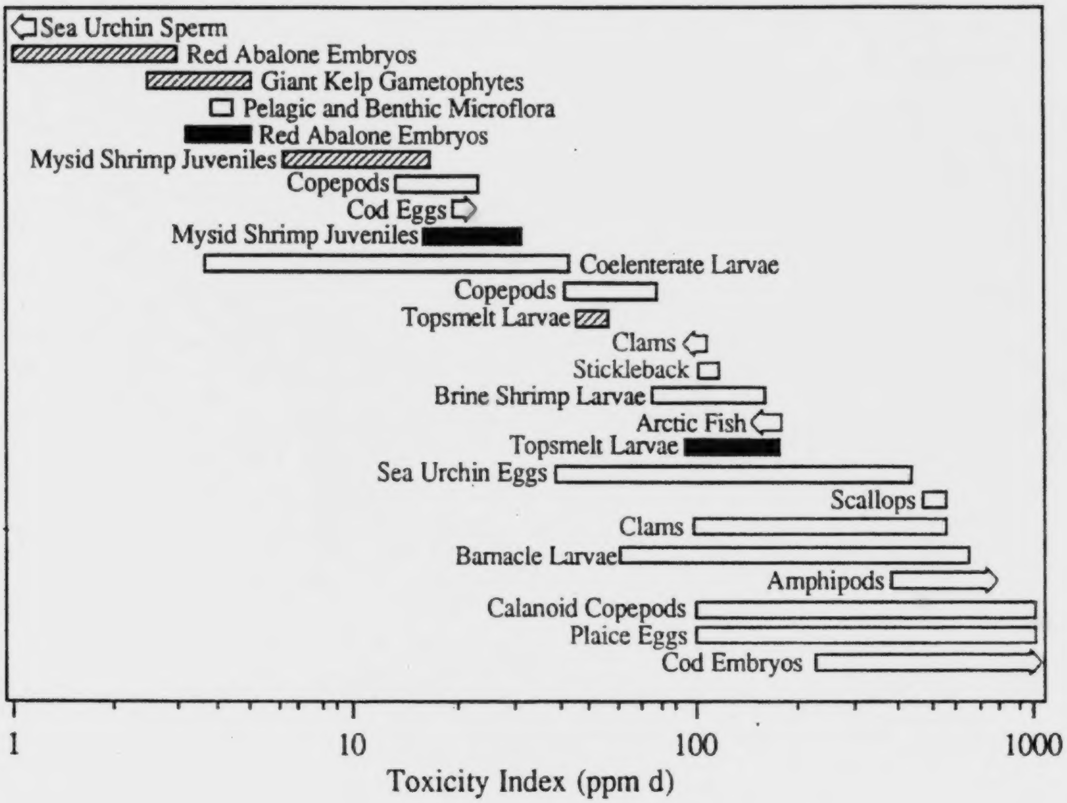

Figure 3. Comparison of results obtained with our system with those of past toxicity studies using Corexit 9527®. Results are presented as toxicity index values (ppm·d), and were calculated from previously published data (Wells, 1984). Crosshatched and shaded symbols indicate NOEC and median effect ranges, respectively, from this study. Arrows indicate toxicity values reported with unspecified upper or lower limits (adapted from Singer *et al.*, 1990b).

three-way stopcock and containing a total of 9 L of solution. At a chamber flow rate of 2 mL min^{-1} (6 mL min^{-1} from each bag pair), stock solutions were replenished every 24 h. The low flow conserved stock chemical, eliminated injury to the test organisms, and reduced the amount of waste effluent. Using the flush time equations derived from Sprague (1969), the flow rate yielded theoretical 50% and 99% chamber turnover times of 1.09 and 7.5 h, respectively, assuring good water quality. If necessary, larger flows can be accomodated with more reservoir bags.

In tests using low chamber flows, both dissolved oxygen and pH levels were maintained well within acceptable limits (Table 1). When testing with Corexit 9527®, which is capable of rapidly depleting oxygen levels in closed systems, concentrations were easily maintained at or above 70% saturation with only minimal manifold aeration. Therefore, complex organic mixtures with low to moderate volatilities may also be reliably tested using our system with aeration. Also, surfactant-based oil dispersants at higher concentrations (100-200 mg L^{-1}) tend to block the fritted disks of the exposure chambers over time. When the chambers were used as part of an automatic dilution system, blockage caused diversion of the various flows, seriously altering the dilution pattern. So,

Table 2. Comparison of NOEC and LC50 values for Corexit 9527® under both spiked- and constant-concentration exposure regimes (from Singer *et al.*, 1990b,c).

Species	Spiked exposure		Constant exposure	
	NOEC	LC50	NOEC	LC50
Red abalones	6.6	15.9	1.1	1.6
Mysids	14.6	140.1	3.3	6.2
Topsmelt	57.0	83.0	13.5	30.7
Giant kelp	~14[a]	92.6	~1.8[a]	NC[b]

[a] Approximate values; one NOEC value less than the lowest test concentration.
[b] NC = not calculated.

when using dispersants, the chambers function best with the design described above.

To evaluate the variation of measured toxicant concentrations from desired nominal levels, water samples were drawn twice daily during the early life stage tests with Corexit 9527® and analyzed at 230 nm with a Perkin-Elmer Lambda II® dual-beam UV-visible spectrophotometer (Norwalk, CT). At dispersant levels ranging from 1-64 mg L^{-1}, variation ranged 5-15% during the 48- and 96-h testing periods; this also includes variability inherent to the analytical technique. Similar results were obtained previously using diluting-type systems (Mount and Brungs, 1967; Garton, 1980; Hong *et al.*, 1987); however, variability may be less when the system is used without aeration, or when using toxicants with less inherent surface activity. Also, tests with Corexit 9527® using our system tended to produce the most sensitive results, indicating that when both biological and chemical factors are maximized, the most conservative information (and most environmentally protective) may be obtained (Fig. 3; Singer *et al.*, 1990b).

Although more expensive than some other systems, ours has several advantages: (1) it allows continuous-flow testing of the earliest, most sensitive, life stages of aquatic organisms; (2) it minimizes degradation of stock chemical during testing; (3) depending upon the toxicant being tested, it may be easily converted between closed and aerated operating modes; (4) it is constructed entirely of inert materials; and (5) water quality parameters may be readily monitored (Singer *et al.*, 1990a). Although our system requires preparation of new stock solutions at regular intervals during a single test, it has the advantage of avoiding variations in toxicant concentrations due to fluctuations in critical timing devices, electric solenoids, siphons, head tank levels, etc. In addition, it may also be used to mimic environmental exposure conditions; by spiking each chamber with an initial dose of toxicant, then allowing clean water to slowly flush it out, single-pulse exposure conditions may be simulated. In previous tests with Corexit 9527®, spiked exposures produced much higher toxicity values, indicating more realistic environmental effects (Table 2; Singer *et al.*, 1990c).

In conclusion, our system represents a significant advancement for use of sensitive microscopic lifestages in aquatic toxicity testing. Also, our design offers an improvement over conventional diluter systems, which may help provide more consistent toxicity data for certain classes of toxicants. Also, water quality problems are mitigated with minimal effect to testing. When using complex organic mixtures or volatile toxicants, our system allows for testing of sensitive life stages in a controlled environment.

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MISE AU POINT D'UNE CHAÎNE TROPHIQUE MODÈLE EN MÉSOCOSMES MARINS POUR L'ÉTUDE DE LA TOXICITÉ SOUS-LÉTALE DES CONTAMINANTS.

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ABSTRACT

SET UP OF A MODEL FOOD CHAIN IN MARINE MESOCOSMS TO STUDY SUBLETHAL TOXICITY OF CONTAMINANTS.

A summary of our research activities in the development of sub-lethal toxicity tests using various cytochemical, biochemical and physiological stress indicators with benthic marine organisms is presented. Some recent results from bioassays performed on blue mussels using methylmercury, dispersed crude oil and PCB contaminated oil are reported. Efforts in developing an experimental marine food chain in the INRS-mesocosm facilities are described. A benthic food chain and a model ecosystem including mussel _Mytilus edulis_, clam _Mya arenaria_, starfish _Leptasterias polaris_, polychaete _Nereis virens_, benthic grubby _Myoxocephalus aenaeus_ and winter flounder _Pseudopleuronectes americanus_ are going to be used for fundamental studies of adaptative mechanisms of benthic species to sub-lethal and chronic environmental stress and for applied works on the development of new toxicity tests. The possible use of mesocosms in the future development of micro-scale toxicity tests is briefly described.

INTRODUCTION

Au cours des dernières années, notre groupe d'écotoxicologie marine a travaillé à développer plusieurs aspects expérimentaux de la recherche environnementale marine dont, en particulier, la mise en oeuvre des mésocosmes capables de recevoir un écosystème marin simplifié permettant l'étude de la toxicité sous-létale des contaminants en milieu semi-contrôlé.

L'objectif de ce texte est de fournir au lecteur un sommaire des problématiques, des objectifs et des méthodologies qui ont été développés par notre groupe et de présenter quelques résultats récents. De plus, le rôle possible des mésocosmes expérimentaux dans le développement de futurs microtests _in situ_ est discuté.

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DÉVELOPPEMENT D'UNE CHAÎNE TROPHIQUE EXPÉRIMENTALE

L'écotoxicologie est une discipline relativement jeune qui a pour principal objectif d'étudier les causes et les effets des stress environnementaux à la fois sur les individus, les espèces et les communautés des écosystèmes naturels. En écotoxicologie aquatique, la notion de stress environnementaux couvre, dans son acceptation la plus large, à la fois les stress naturels engendrés par les variations brusques des conditions physico-chimiques (turbidité, température, salinité) et biologiques du milieu (compétitions intra- et inter-spécifiques, prédation et maladies) ainsi que les stress induits par les activités anthropogéniques comme les émanations atmosphériques acides, les déversements d'effluents industriels, agricoles ou urbains lesquels sont des sources de substances exogènes toxiques (stresseurs) en transit dans les rivières vers les estuaires jusqu'aux océans.

Actuellement, l'énorme complexité des interactions combinées de plusieurs stresseurs sur les organismes vivants qui composent un écosystème interdit une approche holistique (globale) du problème. Malheureusement, la méconnaissance encore profonde des mécanismes biologiques qui soutiennent la survie des espèces et la structure de la communautés, constitue une lacune importante au développement de modèles prédictifs de l'évolution des structures des populations lorsque les fonctions sont affectées par un ou plusieurs stresseurs. Ce manque de connaissances fondamentales limitera considérablement la signification des travaux qui seront réalisés en écotoxicologie dynamique au cours de la prochaine décennie.

L'un des mécanismes fondamentaux de la survie d'un individu et de son espèce est sa capacité aux niveaux moléculaire, cellulaire et tissulaire de tolérer, de supporter et même de s'adapter aux conditions environnementales. Les connaissances sur ces mécanismes ont progressé considérablement au cours des dernières années. Ainsi, on a montré que la synthèse des métalloprotéines, d'abord découverte chez les mammifères et proposée comme protection contre certains métaux toxiques, était de fait présente chez la majorité des poissons, des invertébrés marins et même chez le phytoplancton. De même, l'activation des oxygénases à fonctions mixtes en présence de contaminants organiques est maintenant reconnue comme un mécanisme de protection cellulaire commun à un très grand nombre d'espèces terrestres et aquatiques. Cependant, la relation entre de telles adaptations biochimiques et le développement d'une stratégie procurant des avantages au niveau de la compétition interspécifique dans les communautés reste à démontrer.

L'intérêt de notre équipe de recherche porte précisément sur la découverte d'un ou plusieurs mécanismes de tolérance et/ou d'adaptation communs à plusieurs espèces appartenant à différents maillons trophiques des écosystèmes aquatiques. Des progrès sur les mécanismes de protection aux niveaux hiérarchiques des cellules et/ou des tissus dans un organisme plus complexe, nous semblent essentiels et urgents afin de parvenir à décoder et à interpréter les signaux que nous envoie un écosystème placé sous l'action d'un stress environnemental. On pourra ainsi, à plus long terme, mieux comprendre l'expression structurale des communautés soumises aux stresseurs.

Notre stratégie centrale est la mise en place des différents maillons trophiques expérimentaux en utilisant les 5 mésocosmes expérimentaux de l'INRS-Océanologie.

L'INRS-Océanologie possède à son laboratoire de Pointe-au-Père un groupe de 5 mésocosmes expérimentaux protégés, d'une capacité de 3,5 m^3 chacun qui ont été construits pour des études écotoxicologiques en eaux froides (douce ou saumâtre) et sous un couvert de glace (Pelletier, et al., 1989). Ces réservoirs sont munis d'une paroi double et d'un système de réfrigération permettant de maintenir une température constante malgré les fluctuations des températures extérieures (figure 1). Le sommet de chaque réservoir est exposé à la lumière du jour et à une ventilation forcée capable de générer de petites vagues à la surface de l'eau. Un système de pompage permet de transférer l'eau d'un réservoir à l'autre afin de simuler une dilution progressive d'un milieu contaminé ou de générer une contamination pulsée ou en cascade. Le fond conique est muni d'une trappe à sédiment permettant d'échantillonner les particules tombant de la colonne d'eau. Des échantillons de sédiments et d'animaux benthiques peuvent être déposés sur un support grillagé placé à la base du cylindre. Deux échantillonneurs de 15 cm de diamètre ont été perçés à 1 et 2 m de hauteur d'eau respectivement permettant d'introduire diverses sondes, un diffuseur d'air et collecter les échantillons sans perturber la surface. En hiver, la circulation d'ait très froid à la surface de l'eau provoque la formation rapide d'une couche de glace tout en maintenant la section inférieure du réservoir à une température juste supérieure à la température de congélation de l'eau. Ces mésocosmes servent à développer les maillons trophiques nécessaires à notre étude tropho-dynamique des mécanismes d'adaptation.

Voici un aperçu du programme de recherche en cours:

Première année (1990-91):

a) Détermination des paramètres expérimentaux propres aux mésocosmes protégés (température, lumière, salinité, aération, agitation de surface, mode d'échantillonnage des organismes, système de pompage, contamination pulsée, etc...) dans le but d'établir un écosystème expérimental simplifié mais répondant à des critères scientifiques rigoureux.

b) Expérimentation sur le meilleur choix de l'espèce de poisson à introduire dans l'écosystème expérimental. Les travaux sont en cours sur la plie rouge (Pseudopleuronectes americanus (winter flounder) et le chaboisseau (Myoxocephalus aenaeus) (Grubby).

c) Etablissement dans les mésocosmes des maillons trophiques déjà expérimentés par l'équipe: phytoplancton --- moule --- étoile de mer.

Deuxième année (1991-92):

a) Expérimentation préliminaire sur les indicateurs de stress qui pourraient être communs à plusieurs niveaux trophiques en utilisant d'abord des stresseurs physico-chimiques naturels (salinité, température,...) et ensuite des stresseurs de contamination (métaux dissous et molécules organiques hydrophobes).

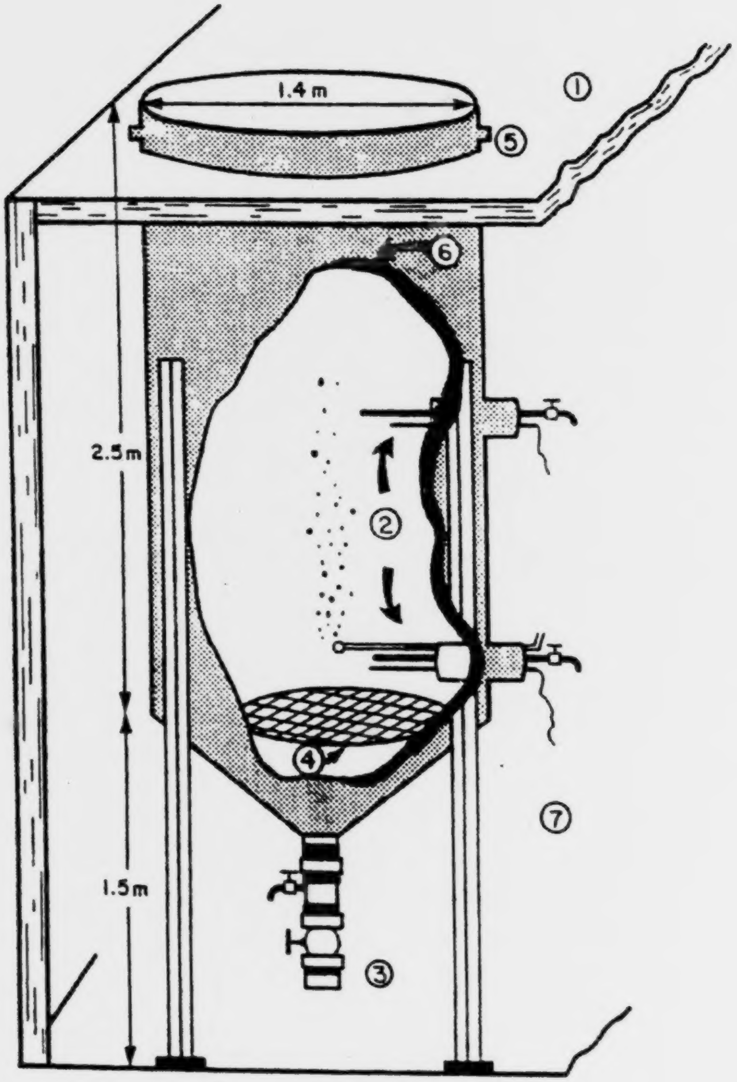

FIGURE 1: Schéma de l'un des cinq mésocosmes expérimentaux développés par l'INRS-Océanologie à Rimouski (Québec).

1: Pont extérieur
2: Échantillonneurs et sondes
3: Trappe à sédiment
4: Grille à benthos
5: Aération de surface
6: Paroi thermostatée
7: Laboratoire intérieur

b) Expérimentation plus poussée sur les communautés phytoplanctoniques dulcicoles et saumâtres avec mesure des indicateurs de stress physiologiques et biochimiques.

c) Poursuite du développement des protocoles d'indicateurs de stress communs pour les organismes pluricellulaires (prolactine, cortisol plasmatique, activités enzymatiques, membranes lysosomales, etc...).

Troisième année (1992-93):

a) Expérimentation simultanée sur tous les maillons trophiques trouvés prometteurs au cours des deux années précédentes.

b) Mesure des indicateurs de stress capables de fournir des informations sur la toxicocinétique de la contamination et sur les mécanismes d'adaptation aux stress environnementaux.

c) Interprétation des résultats par rapport à la problématique générale et aux objectifs énonçés précédemment. Formulation de nouvelles hypothèses de travail.

DÉVELOPPEMENT DES INDICATEURS DE STRESS

L'accroissement constant de l'activité humaine en zones côtières et estuariennes contribue fortement à une augmentation des risques de dégradation du milieu marin. Au fur et à mesure que le développement s'accentue, les conséquences globales finissent par avoir des répercussions beaucoup plus sérieuses que telle ou telle activité en particulier. Les gestionnaires de l'environnement réclament de plus des moyens d'évaluer la qualité du milieu qui tiennent compte des interactions entre les divers contaminants et qui donnent une vision globale du problème. L'approche classique des analyses chimiques du milieu et des essais toxicologiques en laboratoire ne fournit pas ce type d'information.

L' évaluation de la qualité de l'environnement marin côtier devrait donc se faire par le biais de l'étude de l'impact physiologique de concentrations sous-létales de polluants sur plusieurs espèces représentatives de l'écosystème étudié; cet impact étant mesuré par une batterie de mesures physiologiques, biochimiques, cytochimiques et chimiques capables d'évaluer les différents stress environnementaux.

Notre objectif général est de développer et mettre en application une approche écotoxicologique intégrée permettant d'effectuer un suivi environnemental à court et long terme en utilisant plusieurs indicateurs de stress (aussi appelés sondes bioanalytiques) applicables à **plusieurs espèces marines benthiques.** Nos objectifs plus spécifiques sont de trouver de nouveaux indicateurs sur une espèce déjà à l'étude Mya arenaria et Mytilus edulis et les appliquer sur le terrain ainsi que développer des indicateurs nouveaux sur Leptasterias polaris pour les appliquer en mésocosmes expérimentaux.

Les résultats obtenus jusqu'à maintenant (Pellerin-Massicotte, et Pelletier, 1989) montrent dans l'ensemble qu'il est possible d'utiliser les indicateurs de stress comme la fragilité de la membrane lysosomale, l'activité de la malate déshydrogénase, les indices de croissance et de condition physiologique, les concentrations de glycogène, de protéines, de lipides totaux et de polysaccharides avec Mytilus edulis et Mya arenaria à la fois en laboratoire et sur le terrain.

En laboratoire, nous avons testé la sensibilité et la spécificité de la fragilité de la membrane lysosomale et de la malate désydrogénase sur la moule bleue en utilisant les contaminants: méthylmercure, hydrocarbures pétroliers et biphényles polychlorés. La membrane lysosomale est un indicateur ultrasensible à chacun de ces contaminants cependant elle n'est pas spécifique ni à l'un, ni à l'autre. Le temps de réponse est très court dans tous les cas, probablement inférieur à 24 h. La réponse de la membrane lysosomale, quoique intense et très rapide, s'atténue dès la première semaine de la bioséquence de contamination et disparaît généralement en moins de deux semaines. La vitesse de disparition du signal est généralement proportionnelle à l'intensité de la contamination; plus le contaminant est concentré plus la réponse dure longtemps.

Cette méthode des indicateurs de stress a aussi été appliquée à l'évaluation écotoxicologique d'un écosystème que l'on croit contaminé: la baie des Anglais (Baie-Comeau, Québec). Une expérience de transfert à moyen terme (2 mois) de deux bivalves marins, la moule et la mye, a été réalisée entre un site de référence en aval et plusieurs sites présumément contaminés selon un gradient de contamination aux BPC. Les analyses chimiques ont montré qu'il n'y a pas eu d'enrichissement en hydrocarbures, en mercure et en BPC dans les tissus pour toute la durée du protocole cependant plusieurs des sondes analytiques choisies pour évaluer l'état de santé de l'écosystème ont fourni une réponse significative chez la mye (les concentrations en glycogène et en lipides) et chez la moule (la membrane lysosomale). Ces travaux ont montré que l'évaluation écotoxicologique d'un système perturbé peut se faire par l'utilisation simultanée de plusieurs indicateurs de stress sous-létaux (biochimiques, cytochimiques et physiologiques) sur au moins deux espèces importantes (en terme de biomasse) de cet écosystème.

APPLICATION DE LA CHAÎNE TROPHIQUE MODÈLE À L'ÉTUDE DU PROBLÈME DES DÉVERSEMENTS DE PÉTROLE EN MILIEU ESTUARIEN FROID

Malgré un plan d'urgence sophistiqué, l'accident de l'Exxon Valdez, survenu de jour et par mer calme, a causé des dommages environnementaux inestimables au détroit Prince William et Exxon a dépensé 2 milliards pour tenter de nettoyer 1 500 km de plages sableuses et rocheuses. L'estuaire du Saint-Laurent est l'une des régions les plus directement menacées par une telle catastrophe. L'estuaire entre Québec et le Saguenay présente plusieurs similitudes avec le détroit de Prince Williams : chenal de navigation étroit en amont et présence de glace en hiver; présence de nombreux îlots rocheux, récifs et hauts-fonds sableux; présence de mammifères marins et d'oiseaux migrateurs et zone importante de reproduction d'espèces commerciales de poissons et de crustacés.

Au cours de nos travaux antérieurs (1986-1990), nous avons évalué les conséquences écotoxicologiques d'une dispersion de pétrole en eaux très froides (automne et printemps) et même sous la glace. Les principaux résultats de ces travaux montrent clairement que la dispersion chimique, même en eaux glaçées et malgré une efficacité très limitée de la plupart des dispersants, comporte des avantages marqués pour l'estuaire du Saint-Laurent. Les études en mésocosmes ont permis de montrer la dégradation rapide des hydrocarbures dispersés par les bactéries indigènes qui sont très bien adaptées aux eaux très froides. De plus, nous avons observé que le pétrole partiellement dispersé retourne à la surface rapidement mais une grande partie de ses propriétés physico-chimiques a été modifiée et en particulier sa capacité d'adhésion aux surfaces a été considérablement réduite.

Cette réduction irréversible des propriétés d'adhésion du pétrole, que nous avons observée précédemment, avait déjà été rapportée par le Dr Mackay de l'Université de Toronto et avait été attribuée à la formation d'une pellicule rigide autour des gouttelettes. Cette réduction des propriétés d'adhésion du pétrole nous apparaît très importante pour réduire l'impact d'une nappe sur une côte et même sur des animaux marins. Le pétrole traité par un anti-adhésif serait plus facilement nettoyé par les équipes d'urgences et le processus naturel d'auto-épuration serait aussi accéléré.

L'objectif principal de la nouvelle série de travaux que nous entreprendrons en janvier 1991 est de développer un nouveau groupe d'agents chimiques capables de réduire les propriétés d'adhésion du pétrole brut en eaux marines froides (< 10,0 °C) et évaluer leur efficacité et leur toxicité en laboratoire et en mésocosmes.

a) Travaux de toxicité aiguë et de microbiologie

Pour étudier la toxicité aiguë des nouveaux agents chimiques ainsi que du pétrole traité et non traité, nous utiliserons une batterie de trois tests :

1- «Microtox» : test basé sur la bioluminescence de la bactérie Photobacterium phosphoreum et standardisé par l'utilisation du système produit par Microbics Corporation ;
2- «Microalgues» : test mesurant la croissance ainsi que la capacité photosynthétique des microalgues Thalassiosira nordenskioldii (diatomée), Amphidinium carterae (dinoflagellé) et Isochrysis galbana (micro-flagellé), représentatives du phytoplancton du Saint-Laurent.
3- «Artemia salina» : crustacé dont on mesure la survie des individus obtenus à partir d'oeufs incubés (Toxkits).

Le suivi des bactéries sera réalisé à l'aide de tout l'éventail des techniques microbiennes à notre disposition (comptages sur plaques, en tubes (MPN), par épifluorescence, sur milieux spécifiques), parallèlement à des analyses chimiques fines (GC, GC-MS, HPLC) des résidus pétroliers.

b) Travaux en mésocosmes

La figure 2 illustre le protocole expérimental qui consiste à simuler un mouvement de marée diurne dans le premier bassin couplé à une dilution en cascade des eaux contaminées de ce premier réservoir dans le deuxième et le troisième. Le benthos placé sur le support métallique du réservoir 1 sera ainsi soumis à un environnement très semblable à une zone intertidale naturelle.

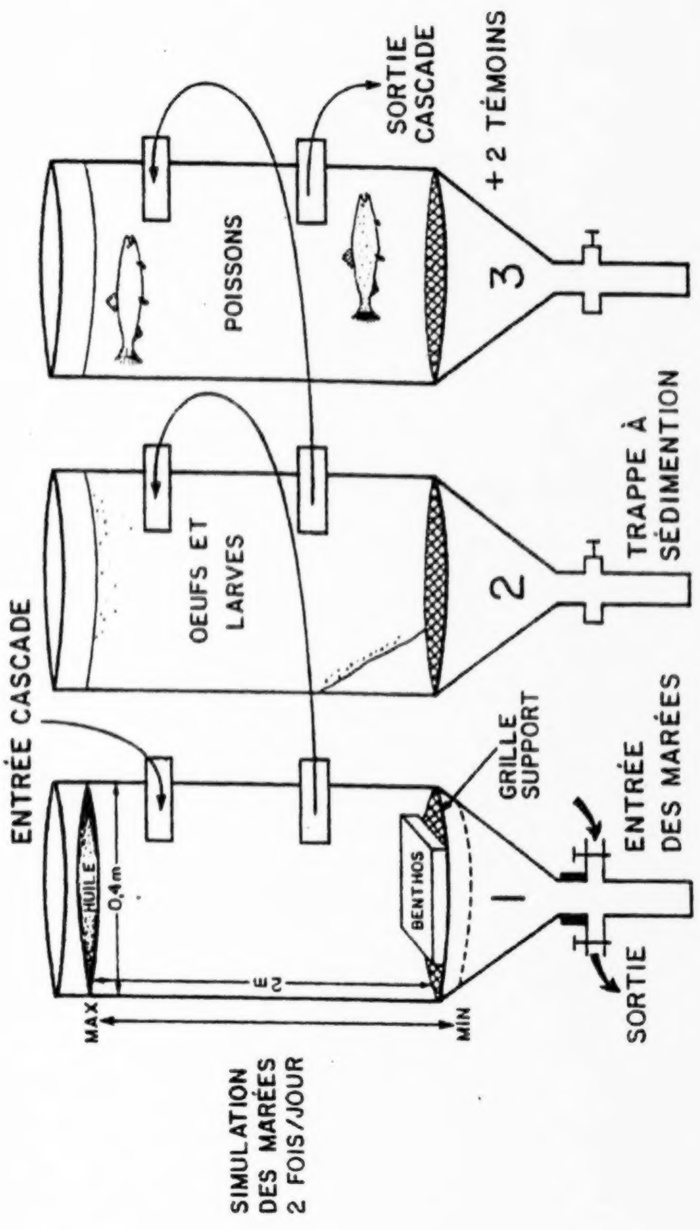
SIMULATION DES MARÉES 2 FOIS/JOUR
MAX
MIN
2 m
0,4m
HUILE
BENTHOS
1
SORTIE
ENTRÉE DES MARÉES
GRILLE SUPPORT
ENTRÉE CASCADE
OEUFS ET LARVES
2
TRAPPE À SÉDIMENTION
POISSONS
3
SORTIE CASCADE
+ 2 TÉMOINS

FIGURE 2: Illustration du protocole expérimental utilisé pour étudier la toxicité sous-létale des agents chimiques de traitement du pétrole.

Le pétrole traité ou non traité sera déposé à la surface du réservoir 1.

Trois espèces benthiques des zones interdidale et subtidale seront étudiées : les bivalves Mya arenaria et Mytilus edulis et le polychète Nereis virens (Sars). Les réponses physiologiques et cellulaires étudiées seront :

1) Évaluation du potentiel de croissance par les mesures de respiration, excrétion et filtration chez Mytilus edulis et Mya arenaria et du rapport O:N chez les trois espèces
2) Évaluation des réserves énergétiques par des mesures de protéines, sucres, lipides et principalement le glycogène;
3) Mesures des activités de la malate déshydrogénase, de la glucose-6-phosphate déshydrogénase et de la pyruvate kinase comme indices de flux de différentes voies métaboliques
4) Mesure de la stabilité de la membrane lysosomale dans les cellules digestives des trois espèces;
5) Calcul des indices de condition physiologique.

Chez les vertébrés, les organismes pressentis sont les oeufs et les larves de hareng et de plie rouge ainsi que de jeunes salmonidés anadromes (omble de fontaine). Les tests effectués serviront à évaluer la toxicité aiguë et la présence d'effets sublétaux potentiels.

1) Survie des oeufs et des larves de hareng et de plie rouge;
2) Taux de difformité larvaire chez les larves de hareng et de plie rouge;
3) Réponse de l'omble de fontaine (Salvelinus fontinalis) au milieu marin en période de migration.

Les oeufs de hareng fécondés peuvent être obtenus du milieu naturel à l'Isle-Verte (50 km de Rimouski) à tous les printemps entre avril et mai. Les oeufs de plie rouge sont fécondés en laboratoire en automne à la Station aquicole de l'INRS. Le chaboisseau pourra éventuellement être aussi utilisé en même temps que la plie.

PERSPECTIVES DE DÉVELOPPEMENT DES MICROTESTS DE TOXICITÉ

Le développement des deux dernières décennies dans le domaine des microtests de toxicité (microscale toxicity tests) a permis d'accroître de façon notable les moyens disponibles pour l'évaluation environnementale à la fois en milieu dulcicole et en milieu marin. L'écotoxicologie marine fait cependant face à un problème très particulier. Contrairement aux milieux lacustres fermés ou semi-fermés, l'environnement estuarien et côtier est ouvert sur l'océan et dispose d'une capacité de dillution qui devrait, en principe, prévenir toute modification environnementale importante. En fait, la plupart des travaux en milieu côtier et océanique montrent que les zones de pollution sont très localisées autour des régions à forte densité de population. Ces zones sont généralement difficiles à caractériser en termes de sources de contamination, de modifications du milieu récepteur et d'effets toxiques; cependant, l'approche de **l'évaluation des risques** fournit une stratégie globale prometteuse qui permet de mieux cibler la contribution possible des tests de toxicité au processus décisionnel.

La quasi-totalité des microtests de toxicité aigüe et sous-létale sont des tests développés pour être réalisés en laboratoire seulement. Cette particularité pose évidemment le problème de l'interprétation écotoxicologique des effets réels dans le milieu mais aussi celui de l'échantillonnage et du soin des échantillons. Ces problèmes sont particulièrement aigus en milieu marin où les volumes sont immenses et la contamination plus diffuse.

Nous croyons qu'il y a présentement lieu d'orienter une partie de la recherche et développement en écotoxicologie marine vers le développement et la mise en application d'une série de micro-tests in situ. Il s'agit d'un défi scientifique et technologique important parce qu'un micro-test in situ devrait à la fois conserver les principaux avantages du microtest de laboratoire (spécificité, sensitivité, répétitivité et faible coût par unité) tout en étant adaptable à un milieu inconnu et non contrôlé. L'utilisation de tels tests, s'ils étaient disponibles, est illustrée à la figure 3. Cette approche est déjà utilisée avec les macro-invertébrés (moules, huîtres,...) qui peuvent être mis en cages et exposés à proximité d'une source de contamination. Cependant, ces travaux avec les macro-invertébrés ont essentiellement porté sur la bioaccumulation à moyen et long terme des contaminants et pas vraiment sur l'évaluation environnementale immédiate. Nous avons besoin de biotests à court terme (24h) capables, en particulier, d'informer l'utilisateur sur les fluctuations toxiques du milieu et sur les contaminations pulsées.

Dans le cadre du développement de tels microtests in situ, les mésocosmes expérimentaux décrits plus haut pourraient devenir des instruments importants en permettant la calibration et la validation des micro-tests dans un milieu semi-contrôlé.

REMERCIEMENTS

Ces travaux de recherche ont été supportés par divers organismes provinciaux et fédéraux par l'allocation de subventions de recherche (CRSNG et FCAR) et de contrats (Pêches et Océans Canada, Environnement Canada et Environnement Québec). Cette publication est une contribution du Centre océanographique de Rimouski - un partenariat de l'INRS (Institut national de la recherche scientifique) et de l'UQAR (Univertsité du Québec à Rimouski) opérant sous les auspices de l'Université du Québec.

OUVRAGES CITES

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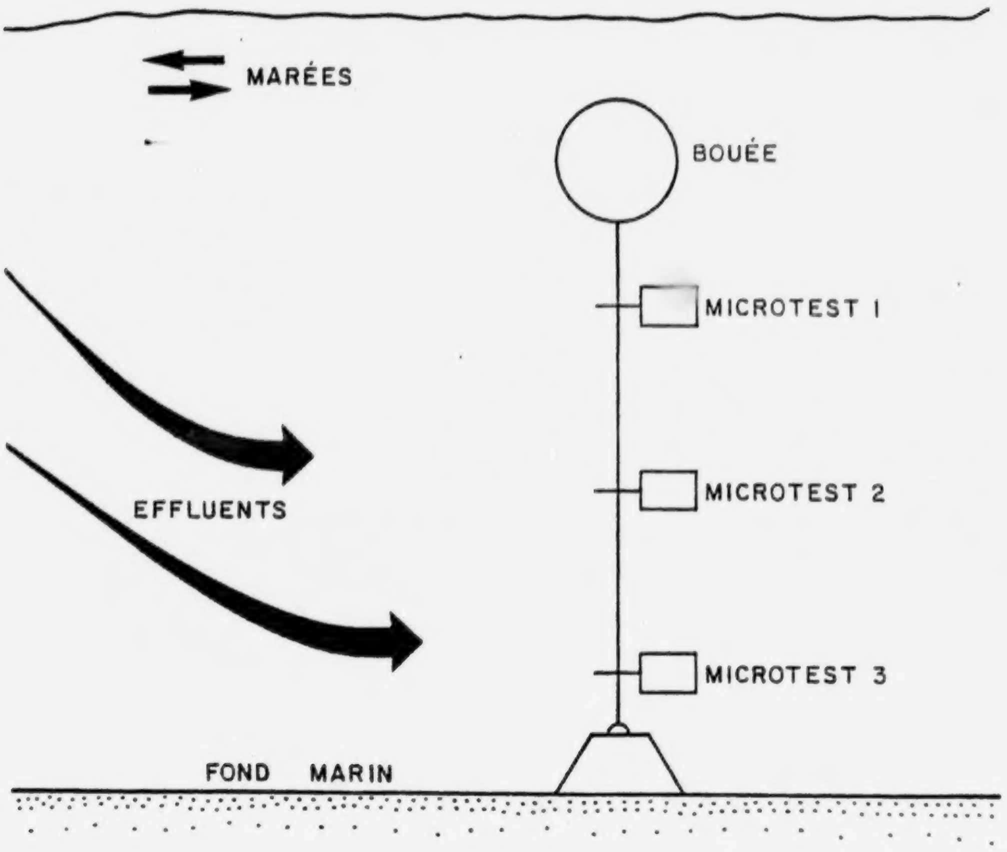
MARÉES
BOUÉE
MICROTEST 1
MICROTEST 2
MICROTEST 3
EFFLUENTS
FOND MARIN

FIGURE 3: Schéma illustrant l'utilisation potentielle des microtests in situ en milieu côtier et estuarien.

CRAB LIMB REGENERATION AND DEVELOPMENTAL TOXICITY

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ABSTRACT

A marine invertebrate model for developmental toxicity testing is described. The regeneration of an autotomized cheliped in the final larval stage (the megalopa) and juveniles of the mud crab, *Rhithropanopeus harrisii*, forms the basis of the model. In its final form, the model will combine a whole-organism assay with *in vitro* toxicity testing; both components will be of short duration. The model is being developed in three phases: In the first phase, the effect of pesticides and a herbicide on regeneration was examined. Certain of these chemicals caused developmental abnormalities. The second phase of the investigation is in progress and will seek to identify, at the cell and tissue level of organization, the developmental process(es) that is disrupted. The final phase will involve *in vitro* mechanistic studies. This paper will describe the current status of the model and its potential for use in aquatic toxicity testing and as a more general model of developmental toxicity.

INTRODUCTION

The purpose of this paper is to evaluate megalopal limb regeneration as a sublethal bioassay of developmental toxicants in the marine environment and as a more general model of developmental toxicity.

A marine developmental bioassay may be applied potentially in three ways: (1) As a sublethal bioassay for toxicants introduced into the marine environment, (2) as a more general model of developmental toxicity and (3) to screen for potential teratogens to higher organisms, including humans. The latter concept is gaining increasing popularity and parallels both the growing interest in marine organisms as models in biomedical research (Marine Life Sciences Workshop 1989) and the search for alternatives to higher vertebrates in toxicity testing (Goss and Sabourin 1985; Goldberg and Frazier 1989).

In recent years, a number of aquatic organisms, vertebrates and invertebrates, have been proposed to test for developmental toxicity (Duffus et al. 1984; Weis and Weis 1987). The embryonic phase of an organism's life cycle is viewed as being particularly sensitive to developmental toxicants. The regeneration of tissues, a process akin to embryogenesis (Weis and Weis 1987), has also been used as a bioassay for teratogens. Particularly noteworthy is the bioassay developed by Weis and co-workers (Weis 1976; Weis and Mantel 1976; Weis et al. 1987a;b) involving the regeneration of an adult crab limb.

Decapod crustaceans have the ability to autotomise their appendages and subsequently regenerate them (McVean 1982). Limb regeneration has received considerable attention and has been reviewed recently by Hopkins (1988). The comparative simplicity of the process and the ability to make non-invasive observations on the process of development make it attractive for studies of developmental toxicity. The regenerated tissues comprise the exoskeleton and underlying epidermis, nerve and sensory tissue (e.g. setae), chromatophores, muscle and blood vessels. The limb develops folded within a cuticular sac; the limb bud. In the adult crab, the limb bud is large enough to permit accurate in situ measurements during regeneration. The relation of the length of the limb bud to the width of the carapace gives an index of regeneration (Bliss 1956). This index has proven useful in assessing the effects of xenobiotics on the regeneration process. In addition to an effect on size, more pronounced effects in the form of malformations have been noted for a range of toxicants (Weis 1976; Weis and Mantel 1976; Weis et al. 1987a;b).

Autotomy is not confined to the adult crab. The final larval stage, the megalopa, also has this ability and is able to regenerate lost appendages which emerge at either the first or second juvenile crab depending on the timing of autotomy and the crab species (Costlow 1963; McConaugha and Costlow 1980; 1987; Clare and Costlow 1989). As the larval stages of an organism are, generally speaking, more sensitive to pollutants than the adult, limb regeneration at the megalopal stage has the potential to be used as a sensitive sublethal bioassay of environmental contamination.

This paper describes the cheliped regeneration bioassay and the effects of 4 pesticides and a herbicide on this process. Some of the preliminary findings from this study have been reported elsewhere (Clare and Costlow 1989; Costlow 1990).

MATERIALS AND METHODS

Source of animals and their maintenance

Adult, gravid, mud crabs, Rhithropanopeus harrisii were collected from plastic crates filled with oyster shells at two sites: The Neuse River and the South River, North Carolina. In the laboratory they were placed individually, together with one oyster shell, into 19 cm diameter, glass, Carolina culture dishes containing 20 ppt, filtered (5um) seawater. The crabs were kept in an environmental cabinet operating at 25°C and a photoperiod of 12h:12h (L:D). The seawater was changed daily, at which time the crabs were fed ad libitum with newly hatched Artemia sp. (Great Salt Lakes). Upon hatching, the larvae were transferred to clean bowls using a wide-bored glass pipette. The larvae were maintained under the same conditions as the adult crabs until they reached the megalopal stage, whereupon, autotomies were performed.

Autotomy

Animals were operated on within 17h of the moult to the megalopal stage. The right cheliped was selected as the limb for autotomy. For the operation, a megalopa was placed on a depression slide and immobilised by pipetting off excess seawater. The merus of the selected limb was then pinched gently with watchmakers forceps. Only those animals that readily autotomised their cheliped were used for subsequent studies (Clare et al. 1989).

Test compounds

All compounds were technical grade and of the highest purity available. Methomyl and RH 5849 were the kind gifts of E.I. DuPont de Nemours Co., Inc. (Wilmington, DA) and Rohm and Haas (Spring House, PA) respectively. Carbofuran was obtained from Chem Service (West Chester, PA) and alachlor and cypermethrin from Crescent Chemical Co., Inc. (Hauppange, NY).

Assay conditions

Range finding tests were performed for all compounds to establish the definitive concentration (nominal) range to be used in the regeneration bioassay. 3-5 replicates of each concentration were used with 6-10 megalopae in each replicate, except for alachlor where a total of 20 megalopae were autotomised at each concentration. Depending on the numbers available, megalopae from one female or a pool from two or more females were autotomised. The appropriate control series was run for each compound: A 20 ppt seawater control was employed for water soluble compounds and an acetone control for those compounds requiring a carrier solvent. Acetone was present in the test and control solutions at a concentration of 1 ppt. Previous studies have established that this concentration of acetone has no noticeable effects on larval development or regeneration.

The autotomised megalopae were placed individually in 15ml capacity glass vials (Fisher Scientific Co.) containing 5 ml of the appropriate solution. The megalopae were maintained in an environmental cabinet at 25°C and a 12h:12h (L:D) photoperiod to the third juvenile crab. Each day, the megalopae or crabs were transferred with a wide-bored glass pipette to clean vials containing freshly prepared solutions to which one drop of newly hatched Artemia sp. nauplii was added. The condition of each animal was monitored daily and the gross morphology of regenerated chelipeds was recorded.

Data analysis

Data expressed as percentages were arcsine transformed prior to statistical analysis. Methomyl data were analyzed as described (Clare and Costlow 1989). G tests for independence were done for the alachlor data according to Sokal and Rohlf (1981). For the remaining compounds, mean survival, duration of development and abnormal regeneration, under the various treatments, were compared to controls by analysis of variance (Sokal and Rohlf 1981). Unplanned comparisons of means were done according to Williams (1971; 1972).

RESULTS

Survival

Based on the results of range-finding tests and subsequent regeneration bioassays, the toxicity of the compounds tested can be ranked as follows: cypermethrin > carbofuran > methomyl > alachlor > RH 5849. Survival in the regeneration bioassay is expressed as the percentage of megalopae that successfully completed development to the third crab stage. For control megalopae, survival is normally in excess of 80% and ideally should approach 100%, although some variation can be expected in the progeny of different females. The effect of the various compounds on survival is presented in Fig. 1. The animals were particularly sensitive to the toxicants at ecdysis from the megalopa to the first juvenile crab (cf. Clare et al 1989).

Duration of development

Protracted development was noted for all test compounds compared to the controls, with the exception of RH 5849. The effect is particularly marked when the cumulative duration of the individual stages of development is calculated, i.e., the period of development from the megalopa to the third crab (Table 1).

Cheliped regeneration

Cheliped autotomy, carried out as described, resulted in comparable survival of operated animals compared to intact controls (cf. Clare and Costlow 1989). It is imperative that the autotomy membrane (Hopkins 1988) is not damaged during autotomy as this membrane prevents loss of haemolymph. Regeneration proceeded in a manner similar to that described for adult crabs (Hopkins 1988). The cheliped developed folded within the limb bud and emerged fully formed, although some two thirds full size, following ecdysis to the second crab stage.

Three of the compounds tested in the bioassay - methomyl, carbofuran and alachlor - caused abnormal cheliped regeneration. For the purposes of this study, abnormal regeneration is characterised as either the failure of a regenerate to appear at the expected ecdysis (second crab stage; Clare et al. 1989) or a regenerate that is smaller than normal and/or malformed. Malformed regenerates ranged from simple stumps with little or no form to chelipeds that were bent the wrong way or which possessed a comparatively minor fault such as a twisted dactyl. Regenerates were not measured; they were compared visually with the contralateral cheliped. The judgement of size was thus rapid but entirely subjective. Fig. 2 shows the incidence of abnormal regeneration for methomyl, carbofuran and alachlor. Regeneration was not affected by RH 5849 or cypermethrin. No malformed regenerates were noted in the control solutions.

DISCUSSION

The two carbamate pesticides, methomyl and carbofuran, and the herbicide alachlor were positive in the bioassay for developmental toxicity at concentrations that were sublethal to mud crab megalopae. Cheliped regeneration in seawater solutions of the synthetic pyrethroid insecticide, cypermethrin and the experimental insecticide, RH 5849 (Wing 1988) was normal at the sublethal concentrations tested. The propensity for developmental abnormalities is not, therefore, a reflection of the compound's toxicity; cypermethrin being the most toxic of the compounds tested and RH 5849 the least toxic. What the compounds do have in common (with the possible exception of RH 5849 which is in the process of evaluation) is that they act as nerve poisons.

The insecticidal activity of methomyl and carbofuran is a virtue of their abilty to inhibit the enzyme acetylcholinesterase that catalyses the hydrolysis of the neurotransmitter molecule acetylcholine (Metcalf 1971). The outcome is a build up of acetylcholine at synaptic junctions and the blockage of synaptic transmission. A reasonable assumption, therefore, might be that the malformations observed in this study are a neurotoxic effect. For example, neuromuscular spasms caused by pesticides are reported to result in developmental abnormalities in fish (Weis and Weis 1987). However, there is reason to believe that this is not so for crab regeneration. First, cypermethrin, also a nerve poison (Narahashi 1986), did not cause developmental abnormalities. Second, other limbs would be affected by muscular spasms (Weis et al. 1987a), yet developmental abnmormalities were only noted for the regenerating limb.

Abnormal cheliped regeneration cannot, strictly speaking, be equated with teratogenesis: The term normally refers to effects on germ cells or embryos. Nevertheless, as has already been mentioned, cheliped regeneration has been likened to embryogenesis. A broader definition of teratogenesis, that would encompass effects noted in this study, has been made by Weis and Weis (1987). Mechanisms of teratogenesis have been summarized by Fishbein (1976) and Weis and Weis (1987) and include: Altered energy sources, enzyme inhibition, mitotic interference, improper cellular communication and are manifested as inappropriate cell death, altered differentiation schedules, inadequate cell migration, etc. It is premature to speculate on the mechanism(s) of abnormal cheliped regeneration, but the result of a vertebrate study on organophosphate and methylcarbamate teratogenesis is noteworthy. Procter et al. (1976) working on the chicken embryo found that teratogenesis did not result from acetylcholinesterase inhibition but from reduced levels of nicotinamide adenine dinucleotide. In this study, carbofuran induced teratogenesis at a dose of 5.0 mg/egg but not at 1.0 mg/egg. Methomyl was only tested at 1.0 mg/egg and at this dose did not induce teratogenesis.

A general effect of xenobiotics on crustaceans is protracted development. Epifanio (1979) suggests that this sublethal effect is a generalised response to stress. The few exceptions that exist (Christiansen et al. 1978; Johns and Pechenik 1980) testify against this theory. In the present study, only RH 5849 did not prolong development. This is not surprising since this compound acts as an ecdysone (arthropod moulting hormone) agonist in insects (Wing 1988) and crustaceans (Clare, Duke University

Marine Laboratory, Beaufort, NC 28516, unpubl. data). On this basis the concentrations of RH 5849 tested in this study may have been too low to shorten development.

Abnormal regeneration and protracted development are sublethal effects at the megalopal and juvenile stages of mud crab development. Nevertheless, the regeneration bioassay in its present from is not meant to be a sensitive indicator of environmental quality. In fact compounds that display sublethal toxicity in the regeneration bioassay are acutely toxic to earlier developmental stages. For example, the 24h EC_{50} for carbofuran to the second zoeal stage of R.harrisii is 9.5 ppb (Clare, unpubl. data). In other words, at the concentrations tested in the regeneration bioassay, the megalopal stage of development would not have been reached.

Cheliped regeneration may be a sensitive indicator of developmental toxicity. We have examined the process of regeneration in the progeny of different females from 2 sites: South River and Neuse River. On the basis of these investigations, comprising observations on some several hundred megalopae and juvenile crabs, it can be concluded that abnormal regeneration does not occur under established optimal conditions of culture. Unfortunately, the data are at present insufficient to make comparisons with other bioassays of developmental toxicity. In particular, further studies on the effect of known teratogens on cheliped regeneration are required.

Several features of R.harissii and the regeneration assay lend themselves to the study of developmental toxicity: 1) The mud crab is a common estuarine organism and has a wide distribution; 2) the biology of R.harrisii has been studied extensively; 3) the adult crabs are easily maintained in the laboratory and optimal conditions for complete larval development have been established (Costlow et al. 1966); 4) although a seasonal breeder in the field, reproduction and development can be manipulated in the laboratory (Bookhout et al. 1984) so that animals are available throughout the year; 5) their small size, especially of the larval and juvenile forms, enables large numbers of known parentage to be maintained so that critical statistical analyses can be performed; 6) rapid development of the larval and juvenile forms means that the regeneration process is complete (i.e. the limb is full size) in approximately 2 weeks at 25°C. Rapid development also means that complete life cycle and multi-generation studies are practicable; 7) crab limbs can be observed by non-invasive techniques; 8) the cheliped, while composed of epidermis, cuticle, muscle, nervous and sensory tissue and the whole perfused with haemolymph, is a comparatively simple structure. Notwithstanding these advantages, the early developmental stages of R.harrisii and other crabs have not been utilized in environmental quality assessment due to a lack of test method standardization (Gentile et al. 1984).

In summary, cheliped regeneration has been used to test the developmental toxicity of a number of compounds. Of these compounds, the carbamate insecticides were the most potent teratogens but the site and mechanism of the developmental lesion were not ascertained. Histopathological studies of cheliped regeneration are now in progress. On the basis of these studies it may be possible to develop a bioassay of developmental toxicity that is sensitive to teratogens, is applicable to the marine environment, especially if used as an in situ test, and may also serve as a more general model of developmental toxicity.

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BIOLOGICAL EFFECTS OF CONTAMINANTS IN HALIFAX HARBOUR SEDIMENT

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ABSTRACT

Halifax Harbour sediments contain heavy metals and organic contaminants. To assess the biological effects of these compounds in the sediment, seven sites spanning a range of known sediment types, water depths, metal and organic contaminant levels were chosen for a series of bioassay tests. Sediment toxicity was measured by the reduction of microbial luminescence in three Microtox tests (pore water, solvent extract and solid phases), by the percent survival in two amphipod species (*Rhepoxynius abronius* and *Corophium volutator*) and by the survival and change in biomass of juvenile polychaete (*Neanthes sp.*). Uptake of contaminants from sediment was assessed by the bioaccumulation test using a bivalve mollusc (*Macoma balthica*). The chronic effects of the sediment on vertebrates were assessed by histopathological studies on winter flounder (*Pseudopleuronectes americanus*) collected from the harbour.

The results of this study were used to evaluate the extent of contamination in Halifax Harbour sediment and to assess the potential value of a battery of laboratory sediment toxicity tests for marine sediment environmental assessment in the ocean disposal program administered by Environment Canada.

INTRODUCTION

For more than a century, Halifax Harbour has been subject to waste discharges from the cities of Halifax and Dartmouth. Untreated sewage discharge, stormwater runoff, river discharge, household and industrial discharges and atmospheric fallout have introduced contaminants into the water column and harbour sediment.

In 1987, a harbour clean-up agreement was signed between Federal Government of Canada and Provincial Government of Nova Scotia for the construction of a primary treatment plant to clean up Halifax harbour. In response to this clean-up project, numerous scientific studies initiated by Environment Canada, Fisheries and Oceans Canada and the Geological Survey of Canada were conducted in the harbour to assess the marine environmental quality of the harbour ecosystem. The results of some of these studies were published in a Canadian Technical Report of Fisheries and Aquatic Sciences (Nicholls, 1989). The report concluded that current state of knowledge of the marine environmental quality of the harbour is limited and that additional studies would be required to enhance our understanding of the harbour ecosystem. However, the harbour sediment chemical data provided by that report have confirmed the apparent polluted condition of the majority of the harbour area.

The present study was designed to assess the biological effects of the contaminants in harbour sediments. Based on surficial sediment chemical data reported by Buckley and Hargrave (in Nicholls, 1989) and Environment Canada (unpublished data), seven sites spanning a range of known sediment types, water depths, metal and organic contaminant levels were chosen for comparison by a series of bioassay tests. Sediment toxicity was measured by the reduction of microbial luminescence of a marine bacterium, *Photobacterium phosphoreum*, in three Microtox tests (pore water, solvent extract and solid phases), by percent survival of two amphipods collected from east (*Corophium volutator*) and west coasts (*Rhepoxynius* *abronius*), and by the survival and change in biomass of juvenile polychaete (*Neanthes sp.*). The bioavailability of contaminants in the sediment were measured by uptake in a bivalve, *Macoma balthica*, and the chronic effects of the sediment on fin fish were assessed by histopathological studies on winter flounder, *Pseudopleuronectes americanus*, collected from the harbour.

The extent of contamination in the Halifax Harbour sediment will be assessed and the sensitivity and potential of each bioassay test conducted in this study will be evaluated to assess their effectiveness for the marine sediment environmental assessment in the ocean disposal program administrated by Environment Canada.

MATERIALS AND METHODS

Field Sampling

A $0.1m^2$ Van Veen Grab was used to collect sediments from the Bedford Oceanography Institute (BIO) research vessel "Sigma-T" at the seven sites (Table 1; Figure 1). Sediments were stored in clean 5 gallon plastic buckets, covered and transported to the Environment Canada Regional Laboratory located at BIO. Subsamples for particle size and chemical analyses were obtained after the sediments were thoroughly mixed in the buckets. The remaining sediment was stored in a refrigerator at 4^oC until used in experiments.

Sampling was conducted at Bedford Bay, Central Bedford Basin, Tuft's Cove, Eastern Passage Treatment Plant Outflow and Drakes Gut on June 28, 1990. These sediments were used for the first set of bioassay tests (Test #1: Microtox Test, Amphipod Bioassay, Polychaete Bioassay and *Macoma* Bioaccumulation Test) which was carried out between July 4 and July 24, 1990.

For the second set of bioassay tests (Test #2: Microtox Test,

Table 1: Coordinates for Sampling Stations in Halifax Harbour, N.S.

LOCATION	LATITUDE	LONGITUDE	DEPTH (m)
1. Bedford Bay	44°43.1'	63°39.9'	12
2. Central Bedford Basin (# 1)	44°41.15'	63°37.6'	44
3. Central Bedford Basin (# 2)	44°41.10'	63°37.8'	60
4. Tuft's Cove (# 1 and # 2)	44°40.65'	63°35.95'	4
5. Imperoyal Jetty (Imperial Oil)	44°38.3'	63°32.8'	12
6. Eastern Passage Sewage Treatment Plant	44°37.7'	63°31.5'	10
7. Drake's Gut	44°36.3'	63°30.35'	5

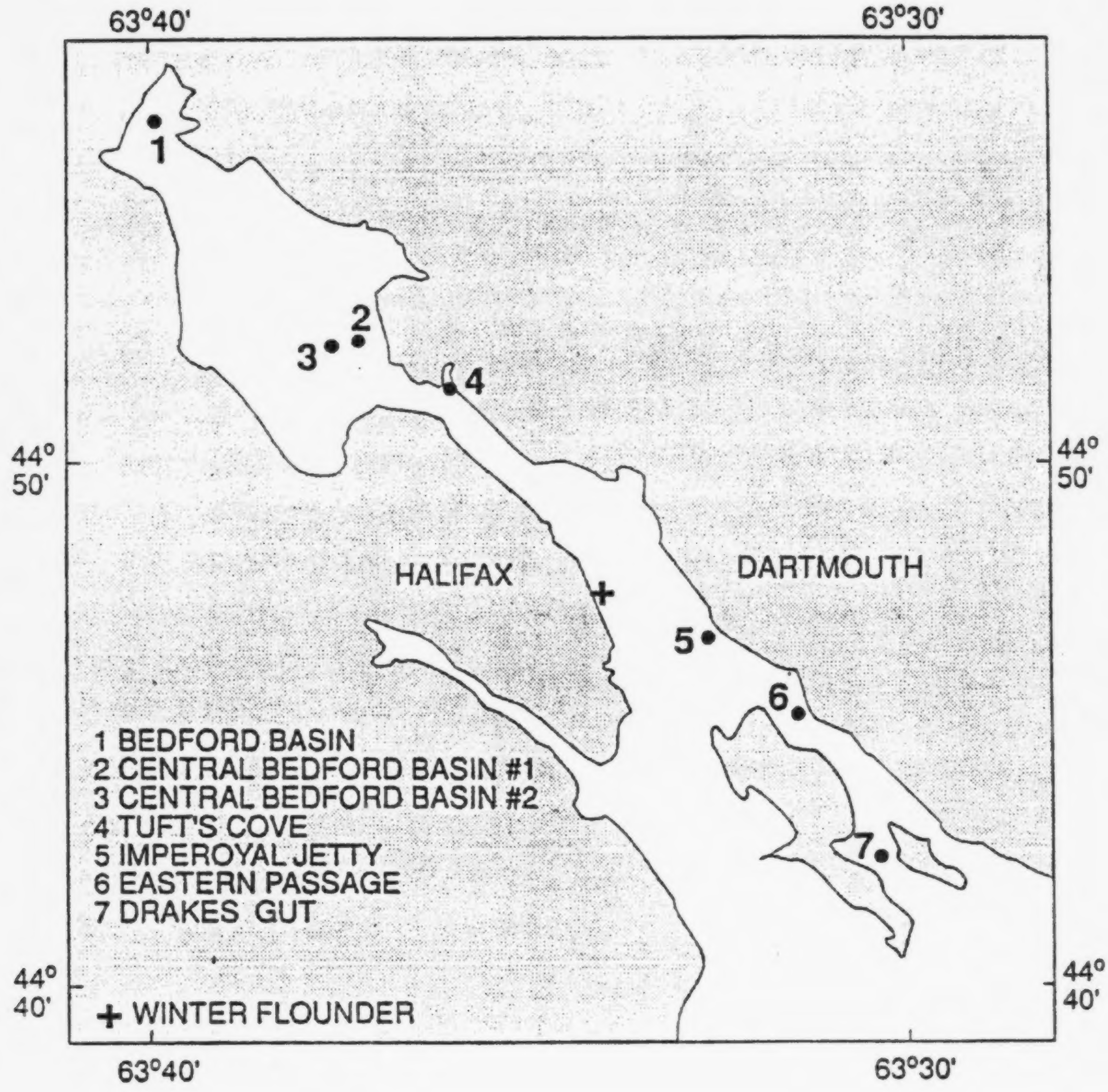

63°40'
63°30'
44° 50'
44° 50'
44° 40'
44° 40'
63°40'
63°30'
1
2
3
4
5
6
7
HALIFAX
DARTMOUTH
+
1 BEDFORD BASIN
2 CENTRAL BEDFORD BASIN #1
3 CENTRAL BEDFORD BASIN #2
4 TUFT'S COVE
5 IMPEROYAL JETTY
6 EASTERN PASSAGE
7 DRAKES GUT
+ WINTER FLOUNDER

FIGURE 1: LOCATION OF SEDIMENT AND WINTER FLOUNDER SAMPLING STATIONS

Amphipod and Polychaete Bioassays), sediments were collected from Central Bedford Basin, Tuft's Cove and Imperoyal on August 16, 1990. These tests were conducted between August 20 and September 12, 1990. In addition to the control sediments, a sediment of known toxicity collected from Sydney Tar Pond, Sydney Harbour, was used in Test #2 to assess the sensitivity of the test organisms.

Sediment Particle Size and Chemical Analyses

Particle size, inorganic and organic carbon analyses were conducted under a contract by Fenwick Laboratories Limited. Particle size analysis was performed by sieve and pipette following the method recommended in the Environment Canada Ocean Dumping Report #1 (Walton, 1978). Total organic matter and inorganic carbon ($CaCo_3$) were determined by loss-on-ignition at 420^0C and 850^0C respectively (McKeague, 1978).

Sediment and tissue heavy metals analyses were performed by the Environment Canada Regional Laboratory. Different digestion methods were used for metal extraction (Cd, Pb, Zn, Cu by total destruction with HF; Hg by sulfuric acid and potassium persulfate; As by sulfuric and nitric acid). Cd concentrations were obtained using flameless heated graphite furnace while Hg concentrations were measured by flameless atomic absorption. Pb measurements were obtained by flame atomic absorption and Zn, Cu and As were measured by ICP atomic emission spectrophotometry (O. Vaidya, pers. comm.).

Polynuclear aromatic hydrocarbons (PAH) were analyzed by both the Environmental Protection (EP) and Inland Water Laboratories of Environment Canada. Analyses of PAH in sediment and tissue samples were conducted according to an in-house EP standard method. Briefly, an undried sediment or tissue sample was subjected to ethanolic - KOH saponification. The diluted digest solution was solvent partitioned and the concentrated extract was eluted from an adsorptive alumina column in order to obtain a PAH fraction. This was further purified by gel permeation chromatography prior to HPLC analysis using external standard techniques. Analytical quality control was ensured by analyzing reagent blanks with each batch of processed samples, by spiked column recoveries, and by replicate analysis of standard reference material (P. Hennigar, pers. comm.).

Sediment Toxicity Bioassays

Three sediment bioassays (Microtox Bioassay, Amphipod Bioassay and Polychaete Growth Test) were selected for this study. The selection criteria were: (1) methods that employed test organisms from different trophic levels (2) test organisms that were sensitive to toxicity of contaminants, easily accessed and maintained in our laboratory; (3) methods employed different end-points for the assessment of biological responses; (4) availabilities of resources and equipment in our laboratory.

For the Microtox Bioassay, we chose the pore water and solvent extract methods which have been used extensively for sediment quality assessment (Schiewe *et. al.*, 1985; Williams *et. al.*, 1986; Ribo and Kaiser, 1987; Giesy *et. al.*, 1988; Ribo and Rogers, 1990; True and Heyward, 1990). We also selected a sediment solid phase method which is being developed by the Microbics Corporation to measure the direct impacts of sediment contaminants on luminescent bacteria (unpublished).

For the Amphipod Bioassay, we chose the West Coast species, *R. abronius*, because a standard method is available for this species. This test has been used extensively on the west coast of the USA for sediment toxicity testing (Swartz *et. al.*, 1982, 1985, 1986, 1989; Chapman *et. al.*, 1984; Williams *et. al.*, 1986; Long *et. al.*, 1990). An East Coast species, *C. volutator*, was used in the same test to compare the sensitivity of the two species.

A polycheate bioassay using survival and change in biomass of juvenile *Neanthes sp.* was selected because polychaetes are one of the major groups of organisms in the benthic infaunal community and the end point used in this test is an important physiological measurement. This test has been developed by the U.S. Army Corps of Engineers and the Puget Sound Dredged Disposal Analysis (PSDDA) study and later adopted by the Washington Department of Ecology to be used as part of the state's marine sediments management program (Johns *et. al.*, 1990).

Microtox Toxicity Tests

Pore water Microtox test

Pore water was obtained from sediment samples by low-speed centrifugation (about 1200 rpm for approximately 45 min). Pore water salinity ranged from 31-34 ppt, close to the Bedford Basin Seawater value of 32.5, with the exception of the Sydney Tar Pond pore water, which had a salinity of 4 ppt. All the pore water tests except Sydney Tar Pond were conducted using Bedford Basin Seawater as diluent (and control), as recommended in the Microbics Methods Manuals (Microbics, 1989a,b). The Sydney Tar Pond sample was ionically adjusted to 22 ppt using MOAS (Microtox Osmotic adjustment solution). One or more potassium dichromate reference toxic tests were conducted with each sample run as a check on technique and reagent sensitivity. In addition, two centrifuged Bedford Basin Seawater blanks were run as a check on the sample preparation method.

Organic Solvent Extract

The method used for solvent extraction was similar to that described by True and Heyward (1990), as modified from Schiewe *et. al.* (1985). The percent total solids of each sample was measured first in order that a consistent quantity of sediment could be extracted. Briefly, to 10 g. (dry weight equivalent) of sample in a 600 ml. glass beaker, sodium sulfate (20 - 200 g.) was added (with occasional stirring) until water was essentially removed. 100 ml. of methylene chloride was then added, and the mixture placed in a sonicator (Branson, model B-S2H) for 15 minutes. The liquid was then decanted off and additional extractions continued until the methylene chloride became clear (two to six times). The combined extract was concentrated to approximately 6 ml. by roto-evaporation in a 35^0C water bath, then transferred to a glass centrifuge tube and further concentrated in a 35^0C bath to 1 ml. under nitrogen gas. One hundred microliters of this extract was added to 3 ml. of ethanol and the solution concentrated to 1.5 ml. (at 35^0C under nitrogen) to ensure total evaporation of the methylene chloride. Ethanol was then added to restore the volume to 3 ml. This was the solution used in the Microtox Test.

Tests on sediment solvent extracts were conducted using a protocol (supplied by Microbics^{TM}) for samples dissolved in organic solvent. The sample was first diluted to 1% in normal microtox diluent, making a 1% sample (10,000 ppm) in 1% ethanol. Further test dilutions were made in 1% ethanol diluent so that all test concentrations contained 1% ethanol. These were run against a 1% ethanol-diluent control which compensated for the slight direct toxicity caused by the ethanol carrier.

All solvent extract samples were first screened for toxicity at 10,000 ppm. The samples were then diluted or concentrated as required to bring them in range and then run according to the standard assay procedure (Organic Solvent Protocol) to determine 5 and 15 min. EC50 values.

One or more potassium dichromate tests were conducted with each sample run as a check on technique and reagent sensitivity. In addition, solvent extract procedure blanks were run to determine if any toxicity was being contributed by the added chemicals (e.g. sodium sulfate, methylene chloride) and glassware. As well, EC50's for double-distilled ethanol were run to determine how much ethanol toxicity the solvent extract procedure was in fact compensating for.

Solid Phase Assay

The solid phase assay was conducted by Microbics Corporation (unpublished method). Essentially, the bacteria are exposed to an aqueous suspension of the whole sediment, the sediment is removed by filtration, and light loss measured using a series of concentrations.

Amphipod Sediment Bioassays

Corophium volutator

C. volutator were collected from the intertidal mud flats at Walton Beach, Bay of Fundy, Nova Scotia. To minimize injury, the animals collected were not sieved out of the mud. Surface mud was placed in water and shaken to release the animals from the sediment. A net was used to catch the dislodged animals.

Animals were transported in 5 - 10 litres of seawater back to the Environment Canada Regional Laboratory. Unsieved sediment was brought back to the laboratory to hold the amphipods. The animals were held in 1x1 metre tanks with flowing seawater and a layer of sediment on the bottom. They were slowly acclimated to 15 + 2°C and held at this temperature for a minimum of five days before the commencement of testing. Excess sediment was brought back to the laboratory and sieved through a 1mm mesh to remove *C. volutator* and other macro-invertebrates. This sieved sediment was used as a control sediment during the toxicity tests.

Rhepoxynius abronius

R. abronius were supplied by EVS Consultants of Vancouver, British Columbia. They were collected from an uncontaminated, sub-tidal site by Whidbey Island, Washington State, United States. After collection, the amphipods were separated from sediment by sieving the sediment along with other organisms and debris and were then reintroduced to the sediment before they were brought to Vancouver in plastic containers. The capped containers were packed in a cooler with ice and flown overnight to Halifax arriving the next day. The amphipods were transported to the Environment Canada Regional Laboratory where they were gradually brought to 15 + 2°C in a continuous flow of seawater. They were held for a minimum of four days at this temperature before the commencement of testing.

Testing Procedures for amphipods

Testings for both species of amphipods were conducted following the Draft Environment Canada Aquatic Biological Test Method "Ten-Day Test for Sediment Toxicity Using the Marine Infaunal Amphipod *Rhepoxynius abronius*" (Environment Canada, 1989).

Five replicates were set up for each sediment tested. Each replicate contained 20 amphipods. The number surviving in the five control sediment replicates was compared with those recorded in the five replicates for each of the other sediments tested on the same date using an ANOVA test in the SAS^{TM} microcomputer program. Significant differences at the 0.05 level between the control and treatment means were calculated by the Dunnett's T Test.

A 96-hour LC50 was performed without sediment for each amphipod species (ten animals per jar) using the reference toxicant cadmium chloride as a check for organism sensitivity.

Polychaete Sediment Bioassay

The Polychaete Sediment Bioassay was conducted following the method described by Johns *et. al.* (1990). Juvenile polychaete, *Neanthes sp.*, were purchased from laboratory stock cultures of D. Reisch, California State University, Long Beach, CA. Five juvenile worms were added to 1 litre glass jars containing 2 cm sediment and filled with seawater (28 ± 2 ppt; 20 ± 1°C). Five replicate jars each containing five worms were set up for each sediment tested. Sieved sand from Whidbey Island, Washington State, was used as control sediment. Each jar was aerated through a glass Pasteur pipette. Eight mg of Tetramarin per animal was added as food every second day and one-third of the seawater was renewed every third day. Worms were exposed to the sediment for twenty days. At the end of the exposure period, worms were removed from the sediments and rinsed with distilled water. All surviving worms from each jar were placed on a preweighed drying pan and dried at 100°C for 24 hours. The animals were removed from the drying oven, allowed to cool in a dessicator, and reweighed. Survival, total biomass (dry weight) and mean individual biomass were the response criteria.

A reference toxicant test was performed without sediment using cadmium chloride. Data were analyzed as for the amphipod tests.

Macoma Bioaccumulation Test

The *Macoma* Bioaccumulation Test was conducted according to a method developed by the Environment Canada Atlantic Regional Laboratory. The test was conducted only in the first set of bioassay tests (Test #1).

M. balthica (Baltic clam) were collected from Walton Beach, Nova Scotia. They were acclimated in sediment collected from Walton Beach and flowing Bedford Basin seawater for 9 days before the commencement of the testing. Sediment from Walton Beach (control), Drake's Gut (reference) and Tuft's Cove were used in the test. Sixteen litres of control water (Bedford Basin seawater) was added to each test tank followed by 4 litres of sieved (0.5 cm mesh screen) sediment. The sediment was then allowed to settle one day (with gentle aeration at 100ml/min) before placing *Macoma* (n = 60) on the surface of the sediment in each test tank. The test was conducted at 15 ± $1^{\circ}C$ for an exposure period of 30 days. Each sediment was tested in duplicate and the tissues were pooled for chemical analysis.

Temperature, dissolved oxygen, pH, and salinity were measured just prior to placing clams in the test tanks and every few days throughout the test period. Observations of clam burrowing and mortality were also made and recorded.

Tissue samples were taken from unexposed clams (n = 120) before the test, and exposed clams at the termination of the test. These clams were placed in running Bedford Basin seawater for 24 hours to allow for depuration of sediment before the tissue samples were removed. Tissue samples were rinsed with deionized distilled water, placed in whirl-packs and glass bottles and kept frozen for chemical analysis.

Winter Flounder Histopathological Study

Three adult winter flounder (*Pseudopleuronectes americanus*) (Total length: 22.5 - 37.0cm; Weight: 152.6 - 437.7g) were caught by fishing line from Queens Wharves in Halifax Harbour (Fig.1).
Fish were held in a flow-through tank in the Halifax Fish Laboratory of the Department of Fisheries and Oceans for approximately two weeks before sacrifice. Length, sex and external abnormalities of each fish were recorded before tissue samples were taken from liver, kidney, gill and gonad for histological examination. Tissue samples (4mm sections) were immediately fixed and preserved in 10% buffered formalin prepared by commercial

source (Fisher). Samples were dehydrated, embedded in paraffin, sectioned at 5 microns, and stained with Harris' haematoxylin and eosin following routine protocols (Luna, 1968). Slides were examined systematically. Nine adult winter flounder collected from Georges Bank were used for control.

RESULTS

Particle Size and Chemical Analyses

Grain size distribution of sediments used in the toxicity tests ranged from the coarse substrate of Sydney Tar Pond sediments (68.6% gravel and 24% sand) to the very fine substrate of sediments from Bedford Bay (55.6% silt and 33.5% clay) and Central Bedford Basin (51.2% silt and 36.5% clay) (Table 2). The Whidby Island sediments where *R. abronius* were collected were mainly sand (98%) while all sediments from the Halifax Harbour with the exception of Drake's Gut were mostly silt and clay. The Tuft's Cove sediments used in Test #2 toxicity test had the highest percentage of clay (52.2%).

Total organic carbon of the tested sediments ranged from 0.77% in the Whidby sediment to 20.3% in the Sydney Tar Pond sample (Table 2). Organic carbon levels in the Halifax Harbour sediments ranged from 3.8% in the Drake's Gut sample to 8% in the second Tuft's Cove sediment sample. The control sediment from Walton Beach contained 3.98% organic carbon which was within the average levels (4.24 + 2.24%) found in Halifax Harbour sediments (Buckley and Hargrave, 1989).

Heavy metal concentrations were high in both the Sydney Tar Pond and Tuft's Cove sediments. High concentrations of Mercury (1.6 - 22.9ppm), Cadmium (0.7 - 2.1ppm) and Zinc (5580 - 13584ppm) were detected in the two Tuft's Cove samples.

Table 2: Particle Size and Chemistry data for sediments used in bioassay tests

Station	As	Cd	Cu	Hg	Pb	Zn	PAH	TIC	TOC	GRAVEL	SAND	SILT	CLAY
	(ppm)							%					
Walton Beach	32	0.1	23	0.04	40	52	0.03	1.13	3.98	0	48.6	34.7	16.6
Whidbey Island	32	0.1	11	0.025	30	40	<0.01	0.24	0.77	0	98	0.3	2
Drake's Gut	15	0.6	25	0.08	46	71	0.77	4.6	3.8	0	51.7	39.8	8.5
Bedford Bay	50	0.4	47	0.32	89	150	0.51	4.6	4.7	0	10.9	55.6	33.5
Ctr. Bedford Basin #1	30	1.4	70	1	173	176	9.2	7.7	4.7	1.7	22.7	50.3	25.2
Ctr. Bedford Basin #2	64	0.1	121	1.6	288	370	8.49	9.13	4.53	1.1	11.3	51.2	36.5
Tuft's Cove #1	50	2.1	243	22.9	297	5580	3.32	19.1	6.4	2.7	21	47.1	29.2
Tuft's Cove #2	35	0.7	402	1.6	265	13584	25.43	25.1	8	4.6	19.2	24	52.2
Imperoyal	34	0.1	53	0.6	134	608	4.37	6.2	4.28	0	13	66.6	20.4
Eastern Passage	25	0.7	65	0.55	103	157	9.41	8.7	4	1	29.8	51.6	17.5
Sydney Tar Pond-100%	19	1.18	104	1.3	570	1625	6615.2	31.1	20.3	68.6	24	3.2	4.1
Sydney: Whidby 1:1-50%	38	0.2	43	0.4	205	595	2538.4	8.96	7.36	12.8	82.8	0.6	3.7
Sydney: Whidby 1:10-10%	37	0.3	16	0.08	58	126	434.8	1.95	2.38	2.5	93.9	0.5	3.1

The concentrations of polynuclear aromatic hydrocarbons (PAH) in Halifax sediments ranged from 0.51 ppm at Bedford Bay to 25.43 ppm at Tuft's Cove #2 station. The two control samples, Walton Beach and Whidby Island, contained 0.03 and <0.01 ppm of PAH respectively. The three Sydney samples contained very high levels of PAH (434.8 -6615.2 ppm) (Table 3).

Microtox Toxicity Assays

In the first set of bioassay tests (Test #1), of the 6 locations studied, inhibition of bioluminescence caused by pore water occurred only in sediment collected from Central Bedford Basin and Eastern Passage (Table 4, Figure 2). However, inhibition caused by solvent extract and solid phase of the sediment occurred in all sediments tested including samples from the reference site. In the second set of bioassay tests (Test #2), with the exception of the pore water test of Imperoyal and Central Bedford Basin sediments, the results showed that all the sediments were toxic to bacteria (Figure 3). Sediments from Sydney Tar Pond and the second Tuft's Cove sample had the greatest inhibitory effect on bacterial bioluminescence.

The control sediments (Walton Beach and Whidby Island) were consistently non-toxic.

Amphipod Sediment Bioassays

The percent survival of both amphipods in the control sediment for the two sets of bioassays ranged from 96% to 100%. *R. abronius* was generally more sensitive to the Halifax Harbour sediments than *C. volutator* (Table 5, Figure 4).

In Test #1, with the exception of the Central Bedford Basin sediment, none of the sediments from Halifax Harbour were toxic to either species of amphipod. The 79% survival of *R. abronius* in the

TABLE 3: CONCENTRATIONS OF PAH (PPM) OF SEDIMENTS USED IN TEST #1 AND #2

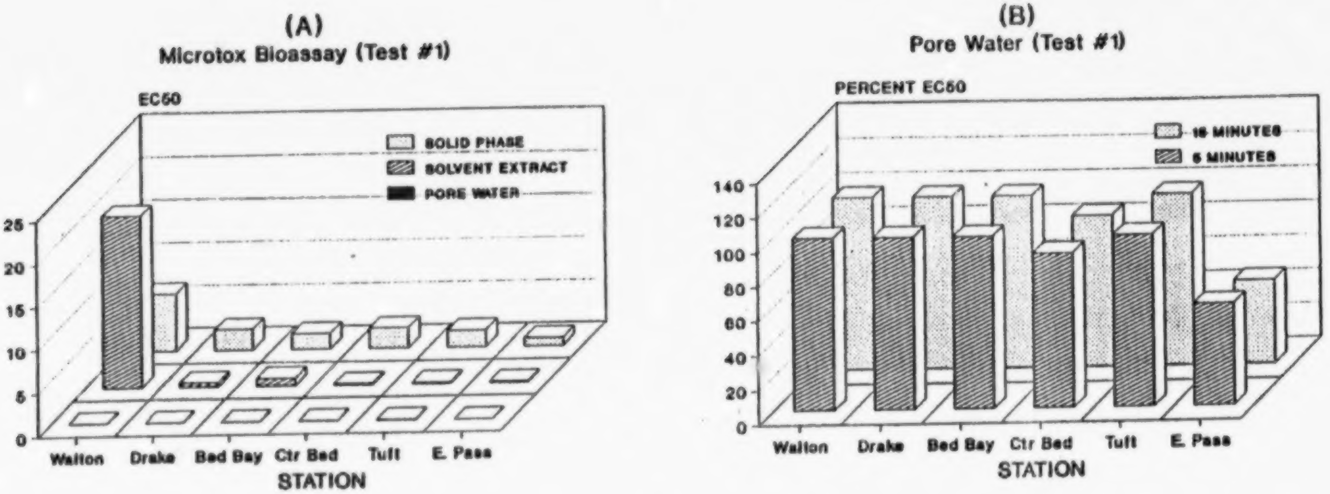
PAH	Walton Beach	Whidby Island	Drakes Gut	Bedford Bay	Ctr. Bedford Basin #1	Ctr. Bedford Basin #2	Tuft's Cove #1	Tuft's Cove #2	Imperoyal	Eastern Passage	50% Sydney Harbour	10% Sydney Harbour
1) LPAH												
Naphthalene	<0.01	<0.01	<0.04	<0.04	<0.01	0.22	<0.04	<0.02	<0.01	<0.2	367	65.4
Acenaphthene	<0.01	<0.01	<0.01	<0.1	<0.05	<0.006	0.07	<0.006	<0.006	<0.06	<0.047	<0.025
Acenaphthylene	<0.01	<0.01	<0.07	<0.7	<0.3	-	<0.09	-	-	<0.4	-	-
Fluorene	<0.01	<0.01	0.09	<0.2	0.19	<0.005	0.06	<0.005	0.19	0.46	85.4	14.6
Phenanthrene	0.02	<0.01	0.07	<0.1	1.1	<0.005	0.23	5.06	<0.004	0.99	484	91.8
Anthracene	<0.01	<0.01	0.03	<0.2	0.37	<0.02	0.08	6.99	<0.01	0.41	253	*IN
2) HPAH												
Fluoranthene	0.01	<0.01	0.11	0.21	1.2	2.32	0.46	4.49	1.13	1.3	386	74.6
Pyrene	<0.01	<0.01	0.09	0.13	1.1	2.53	0.43	1.96	1.45	1.1	330	66.6
Benzo(a)Anthracene	<0.01	<0.01	0.05	0.08	0.54	1.00	0.21	1.16	0.40	0.53	124	24.8
Chrysene	<0.01	<0.01	0.05	<0.2	0.64	<0.008	0.19	3.48	<0.007	0.52	144	34.6
Benzo(b)Fluoranthene	<0.01	<0.01	0.07	<0.09	0.66	0.96	0.36	0.97	0.51	1.1	100	19.3
Benzo(k)Fluoranthene	<0.01	<0.01	0.03	<0.04	0.33	0.18	0.13	<0.001	<0.001	0.6	53.1	10.1
Benzo(a)Pyrene	<0.01	<0.01	0.06	<0.09	0.73	1.28	0.28	1.32	0.69	0.68	116	21.7
Dibenzo(ah)Anthracene	<0.01	<0.01	<0.01	0.05	0.54	<0.002	0.12	<0.002	<0.001	0.41	12	<0.006
Benzo(ghi)Perylene	<0.01	<0.01	0.04	0.04	0.82	<0.005	0.23	<0.005	<0.004	0.46	26.5	<0.19
Indeno(1,2,3-cd)Pyrene	<0.01	<0.01	0.08	<0.2	0.98	<0.005	0.47	<0.005	<0.004	0.85	57.4	11.3
TOTALS:	0.03	<0.01	0.77	0.51	9.20	8.49	3.32	25.43	4.37	9.41	2,538.4	434.8

*IN - Interference

TABLE 4: SUMMARY OF TEST RESULTS FOR THREE MICROTOX SEDIMENT ASSAYS

SAMPLE COLLECTION DATE	STATION	PORE WATER EC50 (%),95%CL		SOLVENT EXTRACT EC50 (ppm), 95%CL		SOLID PHASE EC50 (ppm)
		5 min.	15 min.	5 min.	15 min.	
28 June	Solvent Blank	-	-	>20,000	>20,000	-
"	Walton (Control)	>100	>100	>20,000	>20,000	6728
"	Drake's Gut (Reference site)	>100	>100	474 (115-1948)	400	2460
"	Bedford Bay	>100	>100	867 (555-1352)	820 (574-1171)	1839
"	Central Bedford Basin	~90	~88	154 (83-286)	154	2384
"	Tuft's Cove	>100	>100	192 (72-512)	200	1986
"	Eastern Passage	~59	49 (22-109)	153 (73-320)	164	977
17 Aug.	Solvent Blank	-	-	>50,000	>50,000	-
"	Walton (Control)	>100	>100	-	-	-
"	Whidby Island (Control)	>100	>100	>50000	>50000	>28000
"	Imperial Oil	>100	>100	524 (448-613)	512 (213-1231)	1768
"	Central Beford Basin	>100	>100	162 (119-219)	188 (103-344)	869
"	Tuft's Cove	11.6 (8.2-16.4)	11.8 (2.6-53.1)	58 (52-65)	69 (56-85)	1202
	Sydney Tar Pond	7.4 (5.8-9.6)	8.8 (7.4-10.4)	13.5 (10.3-17.7)	16.8 (12.9-21.7)	1374
	10% Sydney Tar (in Whidby Island)	12.7 (11.1-14.5)	15.2 (12.6-18.4)	261 (224-305)	263 (84-825)	2176

95%CL = 95% Confidence Limits



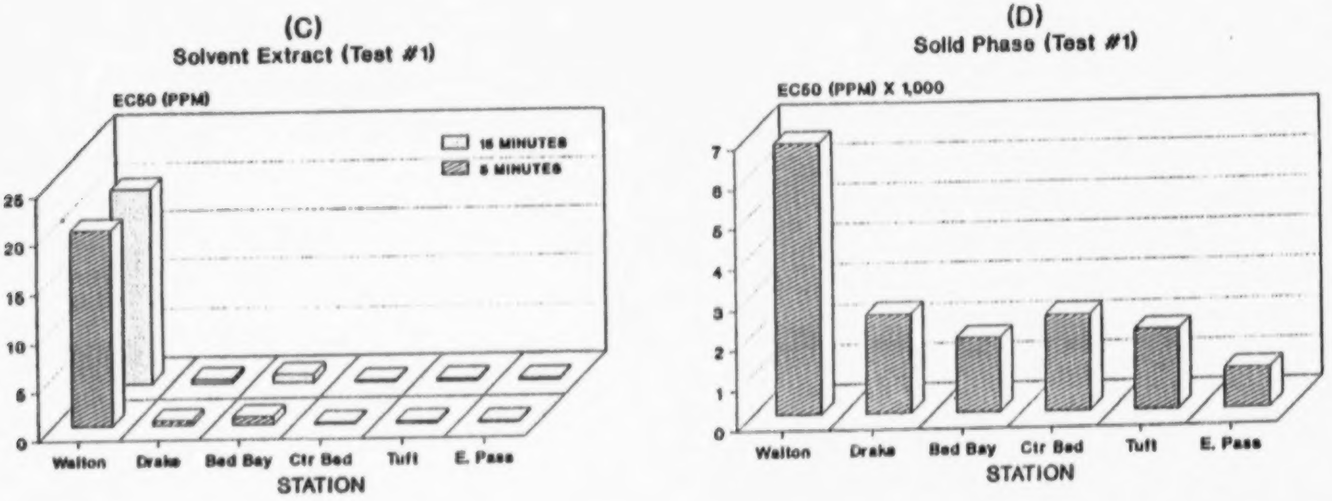
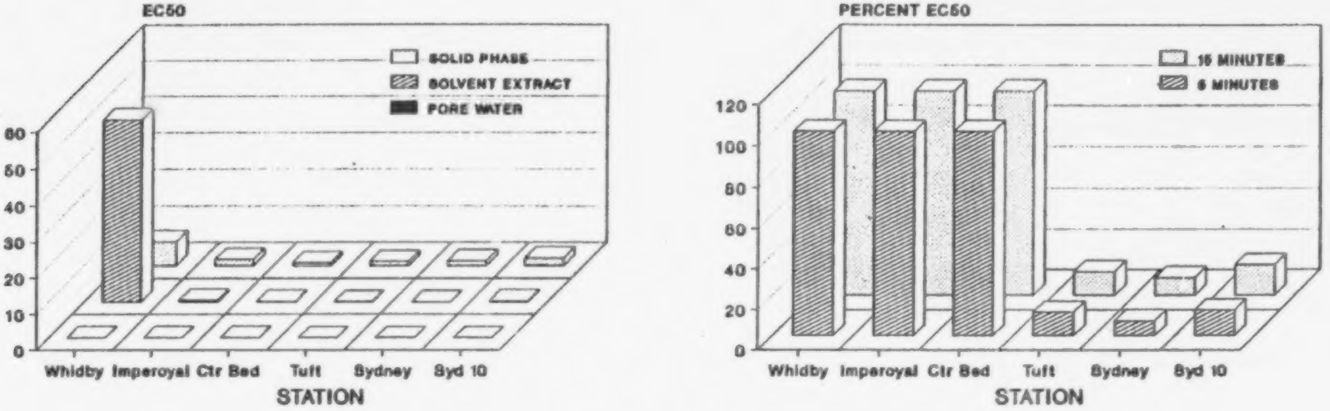


FIGURE 2: RESULTS OF MICROTOX BIOASSAYS IN TEST #1

(A) Microtox Bioassay (Test #2)

(B) Pore Water (Test #2)



(C) Solvent Extract (Test #2)

(D) Solid Phase (Test #2)

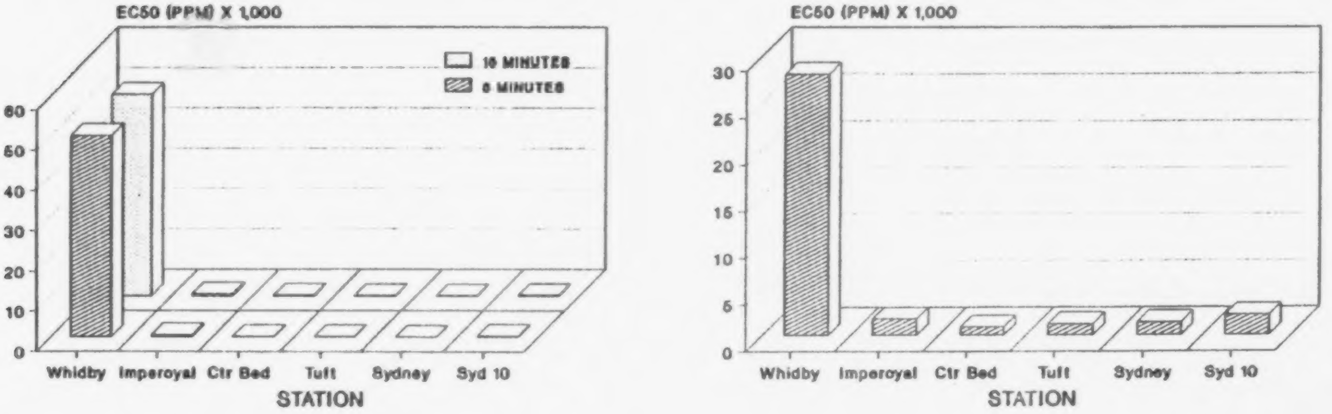


FIGURE 3: RESULTS OF MICROTOX BIOASSAYS IN TEST #2

TABLE 5: RESULTS OF AMPHIPOD SEDIMENT BIOASSAYS

SAMPLE COLLECTION DATE	STATION	COROPHIUM VOLUTATOR		RHEPOXYNIUS ABRONIUS	
		% Survival (N=5)	% Burrowed at end of test	% Survival (N=5)	% Living animals that reburrow in clean sediment within 1 hour
28 June	Walton (control)	97 ± 4.5	Water cloudy	-	-
"	Whidby Island (control)	-	-	100 ± 0	100
"	Drake's Gut (Reference site)	97 ± 4.5	Water cloudy	95 ± 5	95.8
"	Bedford Bay	85 ± 20.6	Water cloudy	93 ± 5.7	92.3
"	Central Bedford Basin	97 ± 2.7	Water cloudy	79 ± 5.5 *	69.4
"	Tuft's Cove	87 ± 9.8	Water cloudy	96 ± 9.0	69.1
"	Eastern Passage	86 ± 8.2	Water cloudy	96 ± 4.1	82.5
17 Aug.	Walton (control)	96 ± 2.2	99	-	-
"	Whidby Island (control)	-	-	97 ± 2.7	100
"	Imperial Oil	99 ± 2.4	Water cloudy	44 ± 7.4 *	90.9
"	Central Bedford Basin	93 ± 5.0	97.3	47 ± 5.7 *	95.7
"	Tuft's Cove	91 ± 7.4	47.3	24 ± 21.0 *	62.5
	Sydney Tar Pond	0 ± 0 *	0 (All dead)	- **	- **
	50% Sydney Tar Pond (in control sediment)	0 ± 0 *	0 (All dead)	0 ± 0 *	0 (All dead)
	10% Sydney Tar Pond (in control sediment)	0 ± 0 *	0 (All dead)	0 ± 0 *	0 (All dead)

* Significant difference ($\alpha=0.05$) from control

** Interstitial salinity was 4 PPT. Rhepoxynius test not suitable at this salinity.

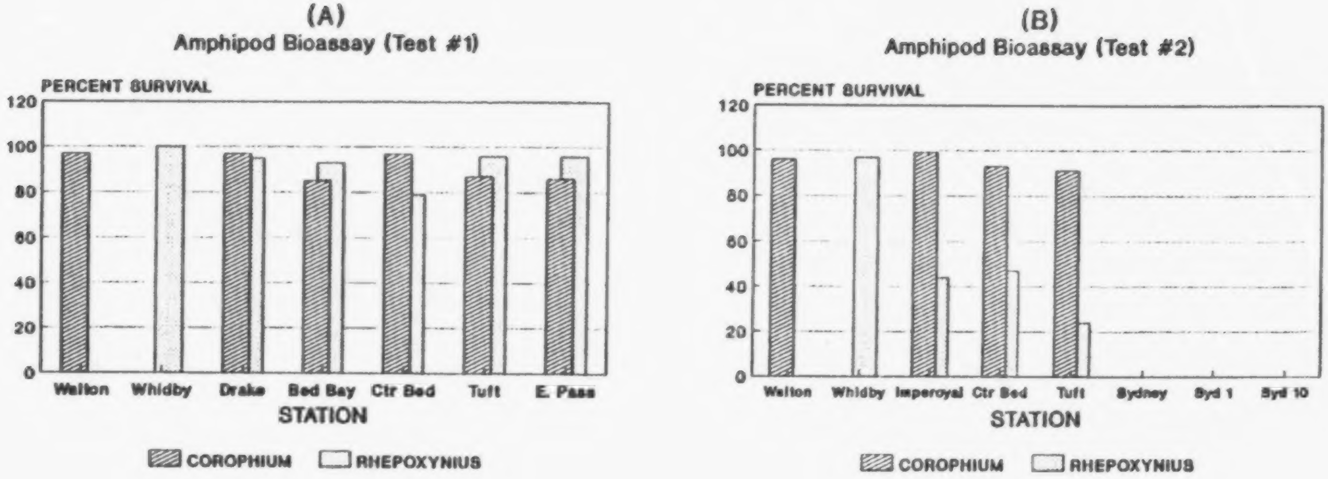


FIGURE 4: RESULTS OF AMPHIPOD BIOASSAYS IN TEST #1 AND #2

Central Bedford Basin sediments was significantly lower than the % survival ($P < 0.05$) in the control batch. The number of *R. abronius* surviving that burrowed in clean sediment in less than a hour after the termination of tests was lowest in the Tuft's Cove and Central Bedford Basin sediments.

In Test #2, sediments from Sydney Tar Pond were acutely toxic to the two amphipod species. Sediments from the three Halifax sites were non-toxic to *C. volutator* but toxic to *R. abronius* ($P < 0.05$). The number of *C. volutator* burrowed in the test sediments at the end of tests (47.3%) and the number of *R. abronius* surviving that burrowed in clean sediment in less than a hour after the termination of tests (62.5%) were the lowest in the Tuft's Cove sediment.

Polychaete Sediment Bioassay

The Halifax Harbour sediments in Test #1 and Test #2 were non-toxic to the polychaete worms in the 20 days exposure experiment. No sublethal effects were detected in all exposures (Table 6; Figure 5). The 50% Sydney Tar Pond sediment was highly toxic to the polychaete larvae. The biomass dry weight measurements of worms surviving in 10% and 50% Sydney Tar Pond sediments (Test #2) were significantly ($P < 0.05$) lower than the biomass of worms surviving in the control and reference sediments.

Macoma Bioaccumulation Test

The percent survival of *Macoma* in the Tuft's Cove sediment was lower than that in the control (Walton Beach) and reference (Drakes Gut) sediments. No uptake of heavy metals and PAH were detected (Table 7, Figures 6 - 7).

TABLE 6: RESULTS OF NEANTHES (POLYCHAETE) BIOASSAYS

SAMPLE COLLECTION DATE	STATION	% Survival (20 days) (N=5)	Average individual dry weight (g) per surviving worm (N=5)	Biomass per Chamber (g) (N=5)
28 June	Whidby Island (Control)	100 ± 0	0.03244 ± 0.00324	0.16216 ± 0.01619
"	Drake's Gut (Reference Site)	100 ± 0	0.03102 ± 0.00403	0.15508 ± 0.02011
"	Bedford Bay	100 ± 0	0.03454 ± 0.00240	0.17266 ± 0.01198
"	Central Bedford Basin	100 ± 0	0.03032 ± 0.00248	0.15156 ± 0.01238
"	Tuft's Cove	100 ± 0	0.03343 ± 0.00336	0.16714 ± 0.01681
"	Eastern Passage	96 ± 9	0.02975 ± 0.00365	0.14174 ± 0.01131
17 Aug.	Whidby Island (Control)	96 + 9.0	0.03150 ± 0.0037	0.1503 ± 0.0161
"	Imperial Oil	100 ± 0	0.03164 ± 0.00251	0.1582 ± 0.0125
"	Central Bedford Basin	100 ± 0	0.02944 ± 0.00504	0.1472 ± 0.0252
"	Tuft's Cove	100 ± 0	0.03114 ± 0.00354	0.1557 ± 0.0177
	Sydney Tar Pond (100%)	- **	- **	- **
	50% Sydney Tar Pond (in control Sediment)	48 ± 46.1 *	0.00874 ± 0.00421 *	0.0224 ± 0.0258 *
	10% Sydney Tar Pond (in control Sediment)	88 ± 17.9	0.01514 ± 0.00511 *	0.0650 ± 0.0232 *

* Significant difference (α=0.05) from control values.

** Interstitial salinity of the Sydney Tar Pond sediment sample was 4 PPT. The Neanthes test is not suitable for this salinity

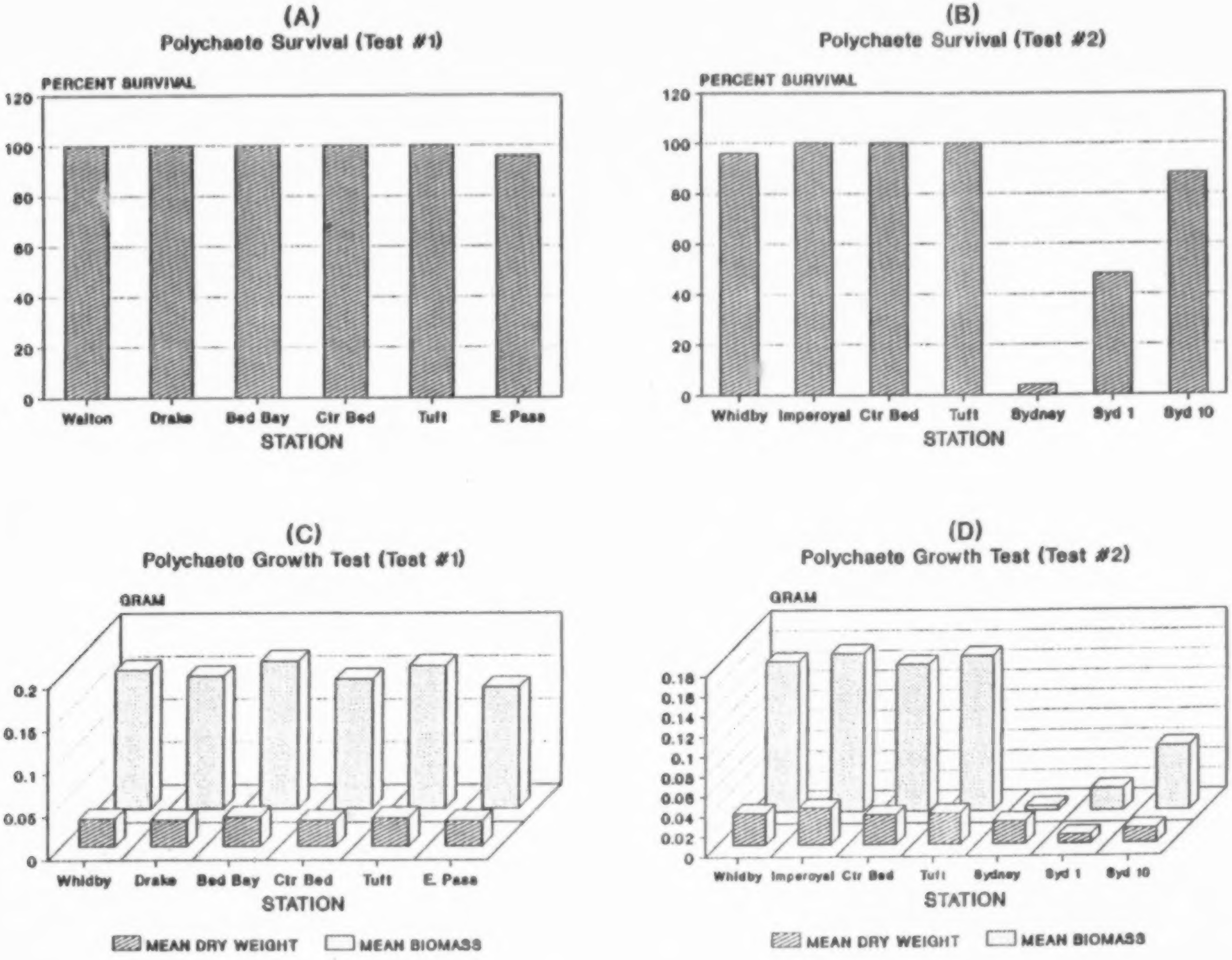


FIGURE 5: RESULTS OF POLYCHAETE BIOASSAYS IN TEST #1 and #2

Table 7: Summary of test results for the *Macoma* bioaccumulation test on Halifax Harbour Sediments

			Contaminants in tissue's (PPM)					
Station	Replicate	% Mortality (in 30 days)	Cd	Cu	Hg	Pb	Zn	PAH
Walton	1	13.3	0.1	7.6	<0.025	9	23	0.11
	2	20.0						
Drakes Gut	1	16.7	0.1	9.2	<0.025	11	28	0.13
	2	18.3						
Tuft's Cove	1	65.0	<0.1	4.6	<0.025	12	25	ND *
	2	73.3						
Original Tissues (Not exposed to test sediments)	-	-	0.2	33	<0.025	12	23	0.01

* ND = None detected - All 16 PAHs below detection limit.

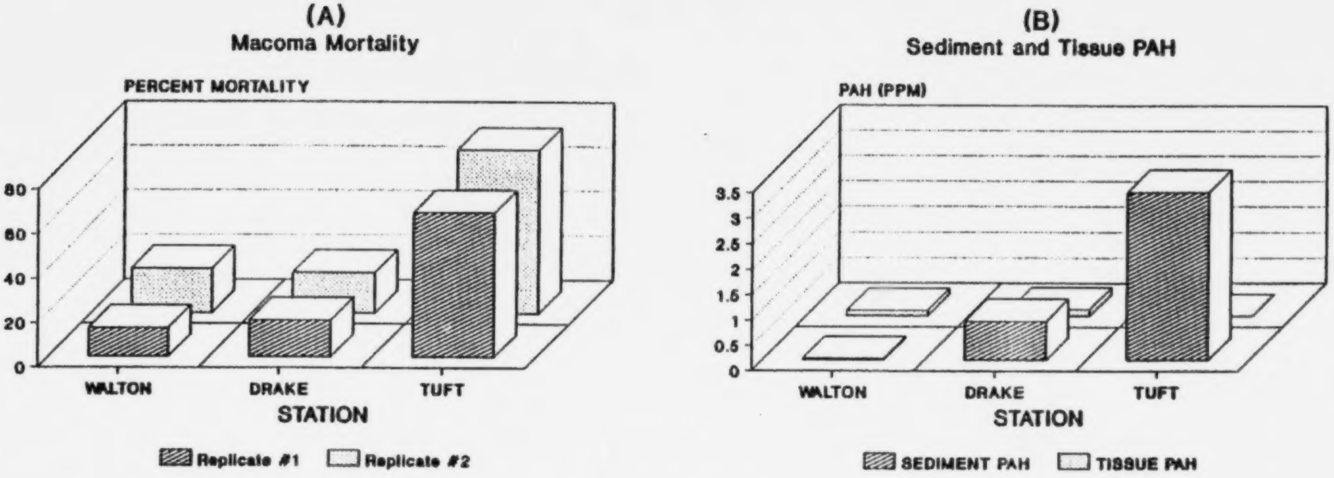
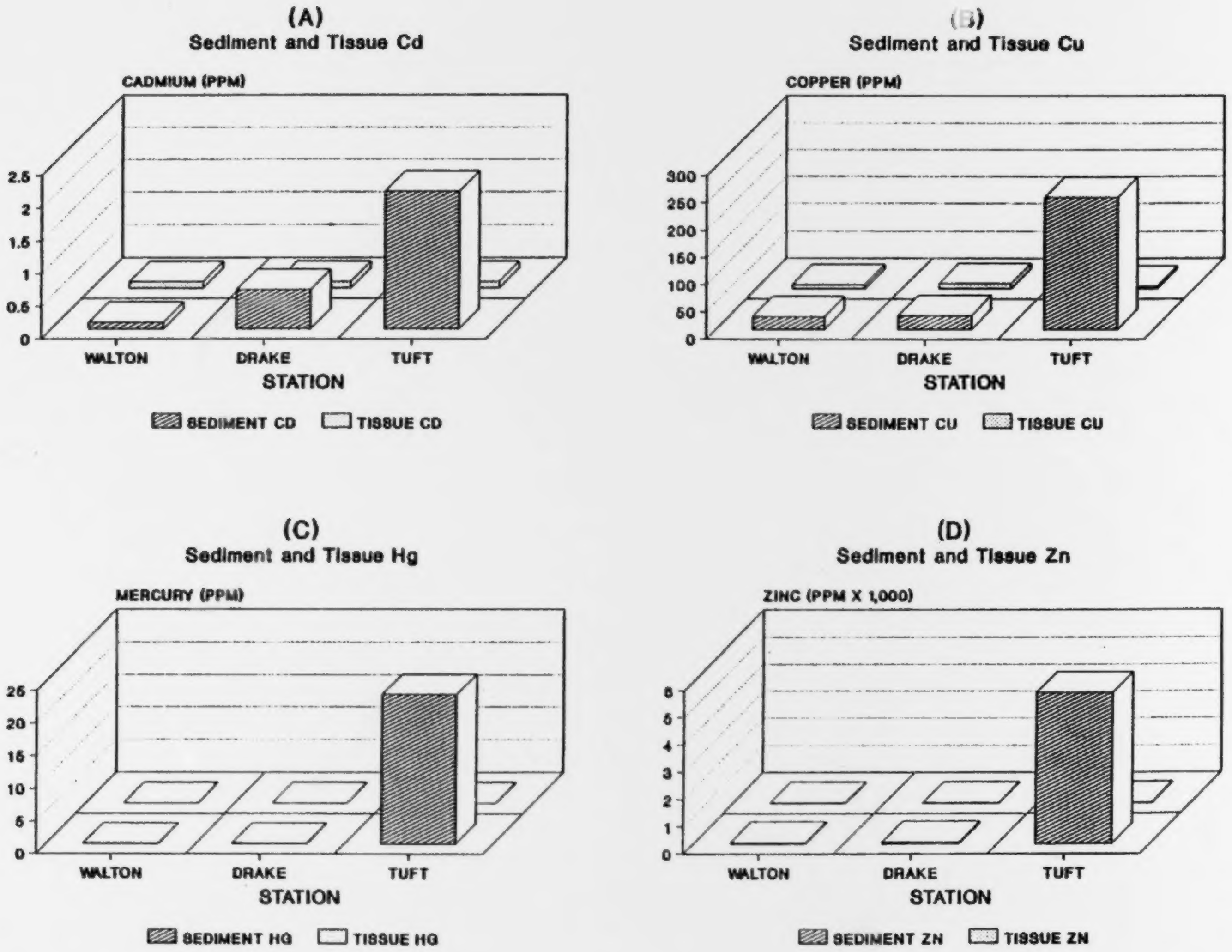


FIGURE 6: (A) PERCENT MORTALITY OF *MACOMA* IN BIOACCUMULATION TEST
(B) SEDIMENT AND TISSUE PAH CONCENTRATIONS IN *MACOMA* AFTER 30 DAYS EXPOSURE


(A) Sediment and Tissue Cd

(B) Sediment and Tissue Cu

(C) Sediment and Tissue Hg

(D) Sediment and Tissue Zn

FIGURE 7: SEDIMENT AND TISSUE HEAVY METAL CONCENTRATIONS IN MACOMA AFTER 30 DAYS EXPOSURE

Winter Flounder Histopathological study

Histological examination of tissue samples of liver, kidney, testis and gills from the nine flounder taken from Georges Bank did not show any lesions.

Macrophage aggregation, hepatic epithelial vacuolation and hepatocyte basophilia were observed in the liver of winter flounder caught in Halifax Harbour (Table 8). The liver parenchyma was often intensely basophilic suggesting low lipid and glycogen stores. Mild early cellular vacuolation was detected, however, no biliary proliferation, vacuolation or neoplasia was seen.

A 1.5 mm focal lesion, with the characteristics of an immature seminoma was present in the testis of one of the Halifax flounder. Numerous spermatogonia were surrounded by nests of poorly differentiated cells.

The gill epithelium was hyperplastic in all Halifax flounder. Normal tubular and hemopoietic structure was seen in kidney of these fish, with many macrophage aggregates present.

DISCUSSION

Contaminants in Halifax Harbour Sediments

Results of the present study indicated that sediments in Halifax Harbour are enriched with heavy metals (As, Cd, Cu, Hg, Pb and Zn), organic carbon and PAH. The levels of Cu (128 ± 130.1ppm), Pb (174.4 ± 97.6ppm) and organic carbon (5.05 ± 1.43%) in sediments collected in the present study were similar to those reported by Buckley and Hargrave (1989). The concentrations of sediment Zn (2587 ± 4821.24ppm) and Hg (3.58 ± 7.83ppm) in our samples were, however, higher than previously found. The concentrations of Zn (13,584ppm) and Hg (22.9ppm) from the Tuft's Cove station were

Table 8: A summary of liver lesions observed in winter flounder from Georges Bank (control) and Halifax Harbour, Nova Scotia

LESION	GEORGES BANK	HALIFAX HARBOUR N.S.
Macrophage aggregation	0/9 *	3/3
Hepatic epithelial vacuolation	0/9	2/3
Hepatocyte basophilia	0/9	3/3

* Numbers of fish with lesions/number of fish examined.

higher than those reported for other North American major harbours, such as Vancouver and Boston Harbours (N.O.A.A. 1988; Goyette and Boyd, 1989; Hubbard and Bellmer, 1989). Buckley and Hargrave (1989) suggested that the contamination sources of Tuft's Cove were derived from mixed sources or were dominated by a specific contaminant in a matrix of sewage waste.

Total sediment PAH concentrations (0.51 - 25.43ppm) of this study were within the concentration range (1.34 - 36.89ppm) reported for Vancouver Harbour (Goyette and Boyd, 1989) but much lower than those (3.25 - 2996ppm) detected in sediments collected from 10 sites sampled within Halifax Harbour in 1987 by Environment Canada (unpublished data). Within the same sampling area located at the Tuft's Cove station, PAH concentrations of 3.32ppm, 25.43ppm (this study) and 2996ppm (1987 Environment Canada unpublished data) were detected in three samples collected on different dates. These data indicated the PAH distribution in sediments from this area is highly heterogeneous.

The patchiness of sediment contamination in Halifax Harbour was expected. The existence of "hot spots" is generally attributed to the integrated effects of the specific location of contaminant sources and sinks, current and sedimentation rates at the local area, degree of wave action, habitat differences of the various infaunal species and physical alterations caused by human activities.

Sediment Toxicity and Sensitivity of Test Organisms

The different biological responses expressed by the four assay organisms (Table 9) were partly due to difference in sensitivities of these organisms to the Halifax Harbour sediments. In the Microtox Test, *P. phosphoreum* was more sensitive to the solvent extract and solid phase sediments than pore water. Similar results were reported by True and Heyward (1990) in a Microtox Test using

Table 9: Association between sediment toxicity and the degree of PAH contamination in sediments. Stations ranked in order based on increasing PAH concentration

Station	Total PAH (PPM)	Microtox			Amphipod		Neanthes sp.		
		Pore Water	Solvent Extract	Solid Phase	C.v.	R.a.	Survival	Avg. dry weight	Biomass
Whidbey Island (control)	< 0.01	N	N	N	-	N	N	N	N
Walton Beach (control)	0.03	N	N	N	N	-	-	-	-
Bedford Bay	0.51	N	T	T	N	N	N	N	N
Drake's Gut	0.77	N	T	T	N	N	N	N	N
Tuft's Cove # 1	3.32	N	T	T	N	N	N	N	N
Imperial Oil	4.37	N	T	T	N	T	N	N	N
Central Bedford Basin # 2	8.49	N	T	T	N	T	N	N	N
Central Bedford Basin # 1	9.2	T	T	T	N	T	N	N	N
Eastern Passage	9.41	T	T	T	N	N	N	N	N
Tuft's Cove # 2	25.43	T	T	T	N	T	N	N	N
10% Sydney Tar Pond (in control sediment)	434.8	T	T	T	T	T	N	T	T
50% Sydney Tar Pond (in control sediment)	2538.4	-	-	-	T	T	T	T	T
Sydney Tar Pond	6615.2	T	T	T	T	NS	NS	NS	NS

C.v. = *Corophium volutator*
R.a. = *Rhepoxynius abronius*
N = Not toxic
- = Not tested
T = Toxic
NS = Test not suitable for this sediment (interstitial salinity = 4 PPT).

interstitial water and solvent extracts obtained from Elliot Bay off Seattle, USA. They suggested that since sediment pore water contained only the water soluble contaminants, their effects on *P. phosphoreum* would only give good estimate of the toxicity of water soluble contaminants. The solvent extract sediment method gives a better estimation of the effects of particle-bound toxins. The combination of endpoints provided by the three phases of Microtox Test used in this study should give a better estimation of the toxicity of both the water soluble and particle-bound contaminants in a sediment quality assessment test. The significantly correlated results (Test #1: $r= 0.97$, $P = 0.002$; Test #2: $r = 0.99$, $P < 0.0001$) of the solid phase and solvent extract methods showed that the solid phase method is a potential tool for marine sediment toxicity assessment.

A significant correlation ($r = -0.94$, $P < 0.01$) existed between the levels of sediment PAH and the toxicity of the pore water exposure. This toxicity did not exist in sediments containing less than 9.2ppm of PAH. The same significant associations between aromatic hydrocarbons and the results of acute toxicity tests in Microtox test have also been reported by Schiewe *et. al.* (1985) and Giesy *et. al.* (1988). Both the results of this present study and those reported by Giesy *et. al.* (1988) suggested that *P. phosphoreum* is generally much less sensitive to heavy metals in the Microtox pore water test.

The relative sensitivity of *R. abronius* to sediment toxicity effects has been repeatedly demonstrated in sediment quality assessment studies on the west coast of the USA (DeWitt *et. al.*, 1988; Long *et. al.*, 1990; Robinson *et. al.*, 1988; Swartz *et. al.*, 1979, 1982; Williams *et. al.*, 1986). Williams *et. al.* (1986) showed that results of Microtox saline extract, oyster (*Crassostrea gigas*) embryo and amphipod (*R. abronius*) bioassays were significantly correlated with one another and concurred in determination of presence or absence of toxic effects in 41% of the test sediment samples. In this study, about 50% of the results of the *R. abronius* tests concurred with the results of the Microtox solvent extract and solid phase tests. In comparison with the control and reference sediments, it is quite clear that the percent mortality of *R. abronius* in Halifax sediments were higher in sediments contaminated with high concentrations of PAH (Table 9). A concentration range of 4ppm to 9ppm will produce variable results while sediment concentration of > 25.43ppm is lethal to the amphipod.

Small particle size and high organic content may sometimes cause or influence the survival of *R. abronius* exposed to uncontaminated sediments (Chapman *et. al.*, 1987; DeWitt *et. al.*, 1988; Long *et. al.*, 1990). We did not find the same effects in this study. While no *R. abronius* could survive in the coarse sediments of 10% and 50% Sydney Tar Pond sediments (2.38 - 7.36% organic carbon; 95.6 - 96.4% gravel and sand), 96% of *R. abronius* survived in the fine substrate from Tuft's Cove #1 sample (6.4% organic carbon; 76.3% silt and clay) and Eastern Passage sediments (4% organic carbon; 69.1% silt and clay).

The endpoint of the *C. volutator* survival test was less sensitive than that with *R. abronius*. We found 91% of *C. volutator* survived the Tuft's Cove sediments which was contaminated by 25.43ppm of PAH, although only 47.3% of the survivals burrowed at the end of the test. The different sensitivities of these two species of amphipod might be due to their different living habitats. *C. volutator* is tube dwelling amphipod (Bousfield, 1973). In comparison with *R. abronius* which is a subsurface burrower, *C. volutator* is frequently exposed to the overlying water as water is pumped through the tube. The limited animal-sediment contact thus reduces the chances of direct exposures of this animal to the particle-bound contaminants in the sediment.

The endpoint of the juvenile *Neanthes sp.* bioassay was less sensitive than that of the Microtox and *R. abronius* bioassays. Correlation Coefficient Analysis indicated a possible negative relation between the concentrations of sediment PAH and the mean biomass of surviving polychaete (r = -0.76, P = 0.08, n=6). The lack of sensitivity of this tube dwelling species to contaminated sediments in this study is quite different from the results reported by Johns *et. al.* (1990). Further evaluation is needed to assess the usefulness of this animal in sediment quality tests.

Bioavailability of Sediment Contaminants in Halifax Harbour Sediments

The uptake experiment in this study indicated that after 30 days of continuous exposure to the Tuft's Cove #1 sediments from Halifax Harbour, no increased concentrations of tissue contaminants were detected in *Macoma*. This result confirms the suggestion in Buckley and Hargrave (1989) that, since most of the organic-rich sediments from Halifax Harbour are in a state of strong chemical reduction, the high concentrations of metals such as Zn, Pb and Hg are probably present as highly insoluble metal sulfides.

The lack of contaminant uptake by *Macoma* in our study might also be due to the following factors: (1) the 30 day exposure time was probably too short for any significant uptake of contaminants in the test animals. Studies conducted by other workers in both laboratory and field experiments showed that uptake of heavy metals (Samant *et. al.*, 1990) and PAH (Malins *et. al.* 1982) occurred after various species of clams were exposed to contaminated sediments for more than 70 days; (2) relatively high levels of organic carbon in the Tuft's Cove sediments might reduce the uptake of contaminants (Landrum *et. al.*, 1987; Swartz *et al.* 1990; Di Toro *et. al.*, 1990); (3) toxic effects of the sediment may have reduced contaminant uptake (e.g. by interfering with feeding).

Winter Flounder Histopathological Studies

Epizootics of hepatic neoplasia in bottom-feeding fish have been described in many areas contaminated by municipal and industrial effluent (Harshbarger and Clark, 1990). In the most comprehensively studied case, that of English sole (*Parophrys vetulus*) in Puget Sound, there is a strong statistical association between the presence of contaminants, especially PAH, and the development of neoplasia (Malins *et. al.*, 1985). These and other contaminants are also suspected to be important in other epizootics of teleost neoplasia.

The results of the present study are preliminary, given the small sample size, but they do suggest that histological changes are evident in winter flounder collected from the Halifax Harbour. The changes seen in the liver of these flounder were mild, but comparable to those reported from Boston Harbour, and other contaminated New England waters. The hepatic changes observed included three of the lesions commonly seen from the Boston Harbour flounder, namely epithial cell vacuolation, macrophage aggregation and hepatocyte basophilia. Additionally perisinusoidal edema was observed in one fish. Epithelial cell vacuolation is a very common lesion in flounder from Boston Harbour, and other heavily contaminated harbours. It is closely associated with neoplastic change, although the precise role of these cells is currently debated (Moore, In prep.). Macrophages aggregate under a number of influences, including chemical contamination (Blazer *et. al.*, 1987). Hepatocyte basophilia is commonly seen in fish from contaminated waters (Moore, In prep.). It reflects a loss of lipid and glycogen stores, and an increase in endoplasmic reticulum and other nucleic acid containing organelles. Perisinusoidal edema is a rare lesion also seen in fish from Boston, and in flounder experimentally exposed to dietary benzo(a)pyrene (Moore, In prep.).

Seminomas are not common. Cases registered at the Registry of

Tumors for Lower Animals include *Zebra danio*, African lungfish, and winter flounder (Harshbarger per. comm.). In a review of fish tumor epizootics (Harshbarger and Clark, 1990) it is suggested that gonadal neoplasms occur in a pattern unrelated to environmental pollution.

Gill epithelial hyperplasia has been described from other contaminated areas. Flounder from Quincy Bay, Boston Harbour, were shown to have partial or complete occlusion of the inter - lamellar space (Gardner and Pruell, 1988). The lesions observed in the Halifax Harbour flounder are primarily limited to basal proliferation. The same basal proliferation was observed in winter flounder fed dietary estradiol (Moore, In prep.). The link between these observations is unclear, but it is interesting that many of the organochlorine contaminants found in coastal sediments are estrogenic (Nelson *et. al.*, 1978), and that estradiol induces respiratory epithelial proliferation in rodents.

CONCLUSION

The results of the present study showed detectable biological effects of the Halifax Harbour sediments on two (*P. phosphoreum* and *R. abronius*) of the four species of benthic organisms tested. Mortality in *Macoma* was increased, although no significant uptake of contaminants were detected in the bioaccumulation test. The hepatic lesions observed in winter flounder collected from the harbour indicated that long term chronic effects of the harbour contaminants were detectable.

The Microtox Test was the most sensitive bioassay for sediment toxicity assessment. The test was most effective for screening the presence of toxic compounds in the sediment when the three phases of test were used concurrently. *R. abronius* was relatively sensitive to the Halifax sediments. For sediments contaminated with intermediate levels of pollutants, the results of this amphipod bioassay were variable. However, because of the relatively high concordance between the responses of this amphipod test and the Microtox test, it was most effective in serving as a supporting study for the Microtox Bioassay. Based on their sensitivities to sediment contaminants observed in this study, we ranked the bioassays as follows: Microtox Solvent Extract > Microtox Solid Phase > *R. abronius* Bioassay > Microtox Pore Water > *C. volutator* and *Neanthes sp*. Bioassays.

For regulatory purpose, such as the assessment of sediment quality for dredged spoils ocean dumping permit applications, where cost effectiveness and efficiency of test are essential for decision making, only the three Microtox Tests and the *R. abronius* are useful in assessing the degree of contamination at the regulatory levels of contaminants. There is, therefore, a recognized need for continuing research and development of toxicity bioassays in regulatory programs such as the Ocean Dumping Program under the Regulations of the Canadian Environmental Protection Act (CEPA). Tests such as the Microtox and amphipod bioassays should be further evaluated with other existing methods, such as the Microbial Enzyme Bioassay (Tay, 1989), Oyster Embryo Bioassay (Williams *et. al.*, 1986), Sea Urchin Embryo Bioassay (Long *et. al.*, 1990), to compare their precision, reliabilities, sensitivities, discriminatory power and ease of use.

The presence of lesions in the few winter flounder examined indicated that, in addition to the lethal bioassays and bioaccumulation studies, chronic effect measurements of sediment contaminants is essential for any pollution control programs such as the Halifax Harbour Clean Up Project. Without collection of baseline data and biological response studies, it is impossible to monitor the results of any pollution control programs which are designed to clean up the marine environment.

The results of this study are preliminary. Because of the limited data set and sampling size for proper statistical analysis and the small number of fish used for histological observation, there should not be any attempt to extrapolate our results and conclusions should not be accepted uncritically.

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PLATFORM SESSION

Methodology & Development I & II

Chair: B. Dutka & A. Farrell

DETOXIFICATION AND RECLAMATION OF SUNCOR'S OIL SAND TAILINGS PONDS.

P.G. Nix

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Suncor Inc. operates an oil sands mining and extraction plant in northern Alberta and utilizes tailings ponds for disposal of waste tailings sludge and for conditioning recycle water. In their active state, tailings ponds consist of a layer of toxic top water overlying a viscous layer of sludge containing toxic interstitial water as well as particles of unprocessed bitumen and clay fines. To address the need for reclamation of the mine site, Suncor is exploring a concept of sludge disposal whereby mature sludge would be pumped out of existing tailings ponds into a mined pit area with an overlying layer of top water. This reclamation strategy would permanently retain both top water and sludge on-site. Its success relies on natural processes of microbial biodegradation and detoxification over time to achieve similar improvements in the quality of the top water layer as could be achieved by more complex waste treatment schemes. Although anaerobic interstitial water in the sludge may remain toxic for long periods, the sludge would be permanently isolated beneath a surface layer of top water. Organic constituents migrating from the sludge layer to the surface water would quickly detoxify.

Toxicological testing has characterized the rate of detoxification and elucidated the nature and extent of subsequent colonization by aquatic plants and animals. The results suggest that tailings ponds can be transformed from toxic waste ponds into viable and productive ecosystems with biologically complex aquatic communities in their surface waters.

INTRODUCTION

The process of bitumen extraction from the Athabasca Oil Sands has resulted in the storage of very large volumes of waste water and sludge in tailings ponds. There are presently four major tailings ponds on the Suncor Lease (Pond 1, 1A, 2 and 3). Their surface area is approximately 240 hectares with the exception of Pond 1A which is about 64 hectares. Each pond shares a basic similarity in that a top water layer (approx. 2 to 10 m deep) with high levels of suspended solids overlies a much denser sludge layer (approx. 40 m deep). This two-phased system of top water and sludge consists of a mixture of waste water, solids, process chemicals, leachates and hydrocarbon wastes. Pond top water is turbid, interspersed with bitumen globules and (in certain ponds) intermittently purged by gases generated within the underlying anaerobic layer of sludge. Sludge consists principally of clay fines and bitumen. Most notably, both top water and water within the underlying sludge layer contain substances which are acutely toxic to aquatic invertebrates and fish.

With respect to eventual mine abandonment, Suncor is required to reclaim tailings ponds to a viable land surface or water body that will be free of long-term maintenance requirements. Since the sludge (approx. 30 - 40% solids) is very difficult to dewater and restore as dry land, one concept for reclamation involves its permanent retention within the area of the Lease by disposal below a layer of top water in a mined pit area. This reclamation scenario is analogous to existing terrestrial programs in which overburden materials are capped with more productive soils such as peat.

Past and ongoing research by Suncor to validate and implement this "wet pond" reclamation concept has as its ultimate goal a reclaimed pond which is environmentally acceptable in all aspects (i.e., water quality, capability to support wildlife and no long term maintenance requirements). The criteria for environmental acceptability are complex and conventional water quality data, utilizing arbitrary chemical and/or acute toxicity tests (e.g., trout LC_{50}), cannot by themselves realistically provide comprehensive environmental assessments of changes in aquatic ecosystems over time. Therefore, as a major component of our research, a suite of toxicological tests was utilized to determine biodegradation rates of toxic compounds within tailings ponds (detoxification) and to predict the character of a reclaimed tailings pond in terms of its biological community structure.

METHODS

In bench-scale tests, 20 L of tailings pond top water was treated to determine if it could be detoxified and to assess the nature and extent of the detoxification process. Top water was treated under varying conditions of dissolved oxygen, temperature and nutrient concentrations (nitrogen and phosphorus). To test the rate of detoxification, five rainbow trout fingerlings (<0.5 g) were exposed to a 3 L sample which was aerated and held at 15°C. The criterion for complete detoxification for these initial tests was the survival of rainbow trout fingerlings for a minimum of 96 h. Other tests utilized 60 L tanks containing varying proportions of top water and sludge.

Pilot-scale tests of Pond 1 top water supplemented with phosphorus (5 mg/L) were undertaken using large steel tanks (9.1 m x 4.6 m) located adjacent to the tailings pond. In 1981, Pond 1 surface water was placed over an underlying layer of sludge in two test tanks with a total volume of 40 m. Tank 2 was filled with sludge from an operational tailings pond (Pond 1) while Tank 4 was filled with debitumenized sludge (i.e., treated Pond 1 sludge). Other pilot-scale tests included two test pits (54 m x 30 m) which were constructed in 1982 and filled to a depth of 4 m with Pond 2 top water only (i.e., no underlying sludge layer) for a total volume of about 6,400 m^3. Pit 1 was supplemented with phosphate to a level of 1 ppm and Pit 2 was left untreated. Toxicological tests were used to assess rates of detoxification on the test tanks (1981-1989) and test pits (1982-1984).

Field tests were also undertaken on a "dormant" pond. For several years (approx. 1977 to 1984), Pond 1A was relatively inactive; that is, there was no continuous discharge of tailings into the pond and any other discharges were incidental and undocumented. Therefore during that period, Pond 1A provided the best available scaled-up model for tailings pond reclamation under worst case conditions (i.e., no treatment of any kind). In addition to direct analysis of pond 1A surface water, the effects of nutrient supplementation on detoxification rates were assessed during the summer of 1981 using water tubes or limno-corrals (approx. 6 m x 1 m) which extended through the surface water layer into the sludge with a total volume of surface water of about 5 m^3.

For toxicological data, one composite sample was collected and replicates used in laboratory testing. Ruth Lake, a nearby shallow and eutrophic lake, was sampled as a reference site and to compare biological and toxicological data with similar data from the various pilot-scale field tests. Trout LC_{50} tests (Standard Methods, 1989) were conducted to determine acute toxicity and to provide baseline data for regulatory purposes in the event that discharge of a treated effluent was considered desirable. However, since predictions regarding the precise extent and nature of detoxification and biological colonization were the focus of this work, more effort was directed toward sublethal and chronic tests using organisms which were representative of various trophic levels in the aquatic food web.

Daphnia magna were tested in accordance with EPA (1987) protocols. Diet consisted of algae (*Ankistrodesmus*, *Chlamydomonas* and *Chlorella*) and trout chow-yeast-cerophyl mix. Twenty-two organisms were used per sample for the 21 d life cycle test. One organism was added to each of seven vessels containing 100 mL of test solution to test for sublethal/chronic effects. Five organisms were added to each of three vessels containing 200 mL of test solution to assess acute toxicity.

Selenastrum capricornutum 96-h bioassay tests were undertaken using a standard protocol (EPA, 1989). One litre of each of the three test samples was filtered through a 0.45 μm pore filter to remove indigenous algae and predators whose presence may alter test results. During the period of logarithmic growth, exponential growth rate constants (Stanier et al., 1970) were calculated.

Trout egg hatchability tests were undertaken using eyed eggs obtained from a local hatchery. Continuous exposure egg hatchability tests were conducted in 2 L glass beakers filled with 1.0 L of test solution. The control consisted of water from Ruth Lake. Twenty eggs were placed in each of five 1.0 L replicates of the test water in incubation baskets made of a glass petri dish (90 mm i.d.) which held the eggs, and a nylon mesh dipnet attached to the dish by silicone, which allowed for easy removal of eggs during mortality checks and solution changes. Test containers were gently aerated, and held at 15°C in the dark to stimulate the hatching process. Eggs and hatched alevins were exposed to light for 1 to 2 h daily during mortality checks and solution changes. Test solutions were changed 3 times per week (95% replacement) and eggs and alevins remained covered with water at all times. Egg mortality, hatchability and alevin mortality were monitored daily. Egg mortality was based on lack of movement and total opaqueness of the embryo. Hatchability was visually observed by the rupturing of the chorion by the embryo and its subsequent survival. Alevin mortality was determined by lack of body movement and heartbeat, body rigidity and opaqueness. Dead eggs and alevins were removed with a wide bore pipette. Tests were conducted until alevins reached swim-up stage (19 d). At termination, wet weight (to the nearest 0.01 g) and standard length (to the nearest mm) and body weight with egg sac removed (to the nearest 0.01 g) were determined for surviving alevins.

RESULTS AND DISCUSSION

Water Quality Characteristics of Tailings Pond Water

Chemical characteristics of all four tailings ponds were similar in many aspects. Levels of composite parameters such as pH (7.9-8.0), conductivity (800-900 μS/cm), and total dissolved solids (1,150-1,490 mg/L) were roughly comparable in all ponds. With respect to dissolved metals/cations, few substantial differences among the tailings ponds were noted. However, Ponds 2 and 3 had higher levels of chromium, cobalt, iron, lead, manganese, mercury, nickel, vanadium and zinc. Top water was acutely toxic with trout LC_{50} values typically in the range of 3 to 10%.

Schram et al. (1984) has suggested that naphthenic acids are the principal toxic organic component in oil sands tailings pond water having a 96 h trout LC_{50} of less than 10 mg/L. These compounds are low molecular weight natural surfactants found in bitumen. Other biodegradable organics which may act as toxicants include phenolic compounds which exist at levels between 0.003-2.5 mg/L and hence may cause toxicity to aquatic organisms (Buikema et al., 1979). Concentrations of ammonia (a biodegradable inorganic compound) in top water range from 2 to 40 mg/L nitrogen. At a pH of 8.0, concentrations of NH_3 (the toxic form) would likely be in the range of 0.1 to 2 mg/L nitrogen and hence likely contribute to a toxic effect *in situ* since 0.41 mg/L value has been reported as the 96-h LC50 for rainbow trout (Hrudey et al., 1976).

Iron in top water exists at widely variable concentrations (0.04-185 mg/L). Since 0.2 mg/L have been shown to be acutely toxic to fish (McKee and Wolf, 1974), iron may therefore exert a toxic effect. The only other metals that were detected in tailings ponds at concentrations exceeding Canadian water quality guideline for freshwater aquatic life (CCREM, 1987) were copper and zinc. Copper concentrations ranged from 0.5 to 4.1 μg/L compared with a guideline of 2 μg/L and zinc concentrations ranged from 7.8 to 50.1 μg/L compared with a guideline of 30 μg/L.

Detoxification

An optimum carbon:nitrogen:phosphorus (C:N:P) ratio for microbial activity is considered to be about 100:33:3 (Atlas and Bartha, 1981). The C:N:P ratio in Suncor's tailings pond top water is about 100:12:0.57 which, by comparison, suggests a possible deficiency in terms of both N and P. Microbial studies using C^{14}-labelled substrates concluded that while non-hydrocarbon compounds (i.e., phenols, glutamic acid) were degraded quickly in oil sands top water, hydrocarbons such as hexadecane and phenanthrene required N and P to stimulate biodegradation (Foght et al., 1985).

In bench-scale studies, aerobic bacterial degradation was shown to be the principal mechanism for detoxification of Suncor's tailings pond top water (Figure 1). Anaerobic conditions resulted in no detoxification (as defined by 100% trout survival over 96 hours) whereas even small amounts of dissolved oxygen stimulated degradation processes. In addition to oxygen, phosphate was required to achieve detoxification within 7 to 10 weeks at 20°C. Temperature effects on detoxification rates were similar to those predicted for bacterial systems; that is, inhibition occurred at 4°C and an approximate doubling in rates was noted for each 10°C increase.

Acute Toxicity

With the exception of 1988, acute toxicity values have been increasing in all tailings ponds since 1981 (Table 1). This trend is most evident for Tailings Pond 1A and likely reflects its return from a semi-dormant pond to an operating pond receiving discharges from the extraction plant. A trend toward increasing toxicity for operating Tailings Ponds 1 and 2 (with the exception of 1988 values) suggest a cumulative effect of tailings discharge resulting in increased concentrations or bioavailability of toxicants over time. In the experimental test tanks and test pits, the reverse trend has occurred (Figure 2). In the test pits, with no underlying layer of sludge, the rate of detoxification was enhanced such that acute toxicity to trout fingerlings was eliminated within two years. In the test tanks, detoxification occurred in three years, likely reflecting the migration of toxic compounds from the underlying sludge layer. In the limno-corrals, a decrease in acute toxicity was noted for those tubes supplemented with phosphate and, to a lesser extent, in Pond 1A itself during the summer of 1981 when there were no tailings being discharged into the pond.

Sublethal Toxicity

Daphnia magna

Untreated tailings pond water was acutely toxic in all cases (e.g., a mean survival time of less than five days). In 1984, after three years of exposure in the test tanks, there was no or little decrease in acute toxicity, and there was no evidence that *Daphnia* could reproduce successfully. However, by 1989 a dramatic shift toward non-toxic conditions occurred. There was no acute toxicity in either test tank and no statistically significant difference ($p \leq 0.05$) in survival between the test tanks and either Ruth Lake water or a laboratory control. Reproductive data indicated that water from both test tanks had substantially less chronic toxicity compared with 1984 test results when *Daphnia* were unable to reproduce (Figure 3). There was no significant difference between Tank 2 and 4 suggesting that the presence of bitumen in the underlying sludge (Tank 2) would not adversely affect processes of detoxification and biological colonization. Water from both tanks did show a significantly ($p \leq 0.05$) lower fecundity compared with water from Ruth Lake, perhaps a result of subtle differences in water chemistry.

Selenastrum capricornutum

Algal growth data for undiluted water samples indicate that all samples (Tank 2, Tank 4 and Ruth Lake) were stimulatory compared to a laboratory control (Table 2). Tank 2 and Tank 4 were also stimulatory when compared to Ruth Lake, likely a reflection of high (5 mg/L) phosphorus concentrations. Exponential growth rate factors (i.e., number of generations per day) were somewhat higher in 1989 compared to 1984 values.

Since Ruth Lake is a moderately eutrophic (nutrient rich) lake, these data indicate that the phosphorus-treated water in the tanks is also eutrophic in terms of its ability to stimulate algal growth. In all cases, the NOEC (No Effects Observed Concentration) was 100%; that is, there was no apparent inhibitory or toxic effect of the test samples on algal growth.

Trout Egg Hatchability

In 1984, trout egg hatchability tests indicated substantial decreases in levels of chronic toxicity to trout in both test tanks and test pits. Compared to Pond 1 water, which completely inhibited the process of hatching, alevins were successfully hatched in both the tanks albeit with a rapid rate of mortality thereafter (Figure 4). These data indicated that although no acute toxicity to trout fingerlings was observed in the test tanks, some degree of chronic toxicity remained. In the test pits, there was almost a 100% success rate in egg hatchability, suggesting that the poorer rate for test tanks was a result of influences from the underlying layer of sludge (Figure 5). In Pit 2, alevin survival was 100% compared to Pit 1 in which all of the alevins died by day 18.

In 1989, there was a trend of increasing success of egg hatchability and alevin survival in both test tanks compared with comparable tests for Ruth Lake water (Table 3). At day 19, when the alevins were approaching the swim-up stage, the fish were sacrificed and the weight of the egg sac was recorded as a percentage of total body weight. These data reflect the ability of the fish to convert the egg yolk into energy for growth and are considered a sensitive indicator of toxic effects since the alevin life stage is most susceptible to the impact of toxic compounds. If the yolk sac is small in relation to total body weight (i.e., a smaller percentage), then more energy has been transferred to the alevin and there is less indication of any toxic effect. The data showing predicted body weight (average for control fish) less the mean for a specific sample indicate that the water in both test tanks was significantly ($p \leq 0.01$) more toxic compared with Ruth Lake in terms of sublethal effects on alevin growth. And, as indicated by alevin mortality, the data suggest that Tank 2 water had a greater sublethal toxic effect compared with Tank 4.

Toxicology of Sludge

An important issue in the toxicology of a reclaimed sludge pond is contaminant behaviour in the sludge layer. The sludge layer is the most dominant aspect, by volume, of tailing ponds fluids. Only recently has the need for development of criteria to evaluate contaminant levels in sediments (i.e., sediment quality criteria) been addressed by regulatory agencies and institutions. EPA and Environment Canada are evaluating several approaches, but it will be several years before guidelines, let alone criteria, are set up.

The details of toxicant transport, distribution and accumulation in sediments are extremely complex and not amenable to simplistic evaluation. Contaminants in sediments have a wide range of availability which depends on chemical and biological conditions, physical properties of the sediments and the nature of the contaminants (Jennett et al., 1980). Despite these difficulties, it is important to assess sludge toxicity since sediments/sludge solids can function as a source of contaminants to unpolluted overlying water and/or groundwater.

Pilot-scale studies using test tanks suggested that detoxification is inhibited, but not prevented, by the presence of an underlying sludge layer. Laboratory studies using 60 L tanks with tailings pond top water overlying various amounts of sludge (0% and 90% by volume) showed that some detoxification of the top water occurred over a period of about 10 weeks (Figure 6). However, the detoxification rate was inhibited and CODs and TOCs were increased in tanks containing top water overlying a sludge layer. Since factors which might affect bacterial degradation rates (e.g., pH, temperature, dissolved oxygen, phosphate levels) were comparable in all tanks, it was concluded that inhibition of detoxification in those tanks with an underlying sludge layer occurred as a result of the migration of unidentified toxic chemicals from the sludge layer into the top water.

It is likely that both the physical (i.e., viscous) and chemical (i.e., toxic interstitial water) characteristics of sludge will inhibit colonization by benthic organisms. In addition, elevated concentrations of toxic compounds in sludge water may have a chronic toxic impact on the survivability of animals which utilize the sludge-water interface in early life stages (e.g., certain species of invertebrates and fish).

SUMMARY

Tailings ponds have the capability of undergoing transformations that remove toxicity and allow maintenance of biologically complex aquatic communities. Aquatic organisms in reclaimed ponds would be expected to include phytoplankton, invertebrate animals and fish. In view of the lack of field-scale data, further work is required to confirm this conclusion and to more accurately estimate the degree of environmental acceptability that is possible under various design scenarios for a reclaimed pond.

ACKNOWLEDGEMENTS

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Table 1. Acute toxicity data for tailings ponds, test tanks and test pits, 1981 to 1989.

Sample I.D.	96-h LC50 (% vol/vol)				
	1981	1982	1984	1988	1989
Tailings Pond 1	17	7.5 - 10.2	4.5	28	3.2
Tailings Pond 1A	27	24	5.8	92	3.2
Tailings Pond 2	16	4.2 - 5.1	4.2	26	3.2
Tailings Pond 3	-	-	-	5.8	3.2
Tank 2	13	35	>100	-	>100
Tank 4	22	17	45	-	>100
Pit 1	-	1.7 - 13.5	>100		
Pit 2	-	8.4 - 20	>100		

All data taken from E.V.S. reports with the exception of 1988, which was taken from Suncor in-house reports.

Table 2. Growth response and exponential growth rates of *Selenastrum capricornutum* to undiluted samples of water from the test tanks and test pits, 1984 and 1989.

Sample	Exponential Growth Rate (k)		Growth Response Compared to a Laboratory Control	
	1984	1989	Percent Inhibition	Percent Stimulation
Ruth Lake	1.2	1.6	-	67
Tank 2	0.95	1.8	-	201
Tank 4	1.4	1.8	-	191

Table 3. Assessment of chronic toxicity in test tanks using the rainbow trout egg hatchability/alevin survival test, 1984 and 1989.

Sample	1984	1989
Percent Egg Hatchability		
Tank 2	82	91
Tank 4	80	86
Ruth Lake	92	90
Percent Alevin Survival		
Tank 2	0	43
Tank 4	0	50
Ruth Lake	77	67
Body Weight (mg) (Predicted - Mean)		
Tank 2	-	-12.1
Tank 4	-	-3.8
Ruth Lake	-	+28.9

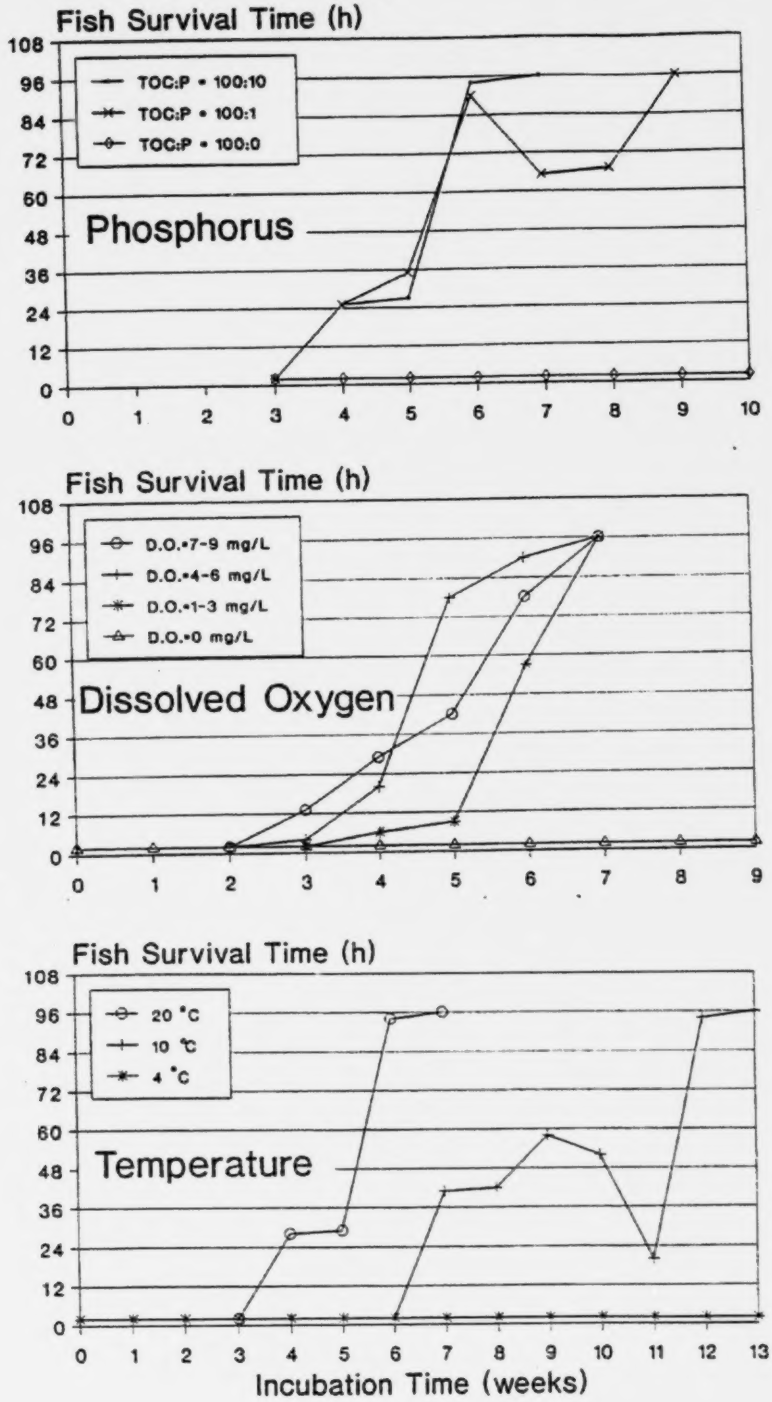

Figure 1. Detoxification of tailings pond top water in the laboratory under varying conditions of phosphorus supplementation, dissolved oxygen and temperature.

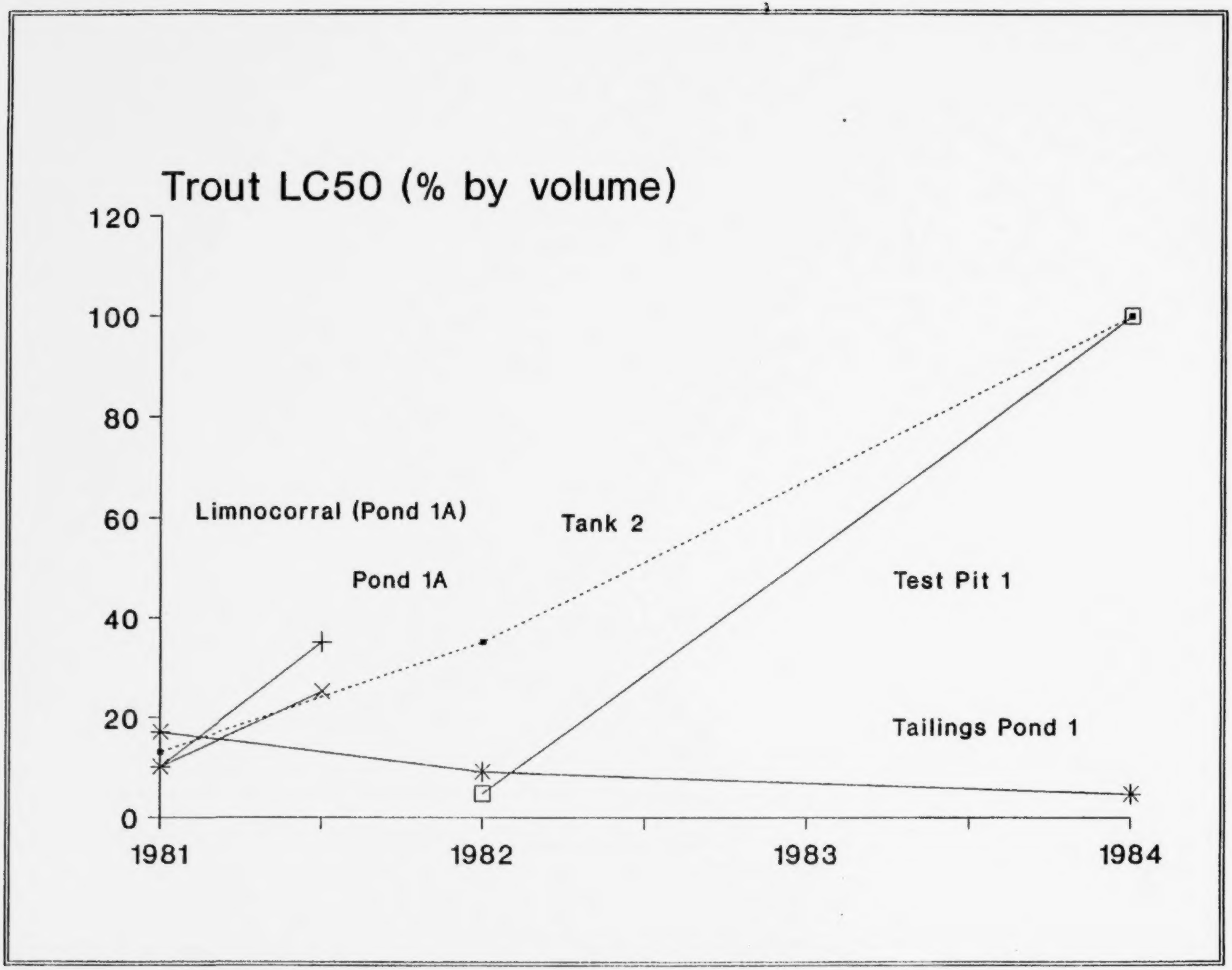

Figure 2. Detoxification of tailings pond top water in pilot-scale experiments in the field.

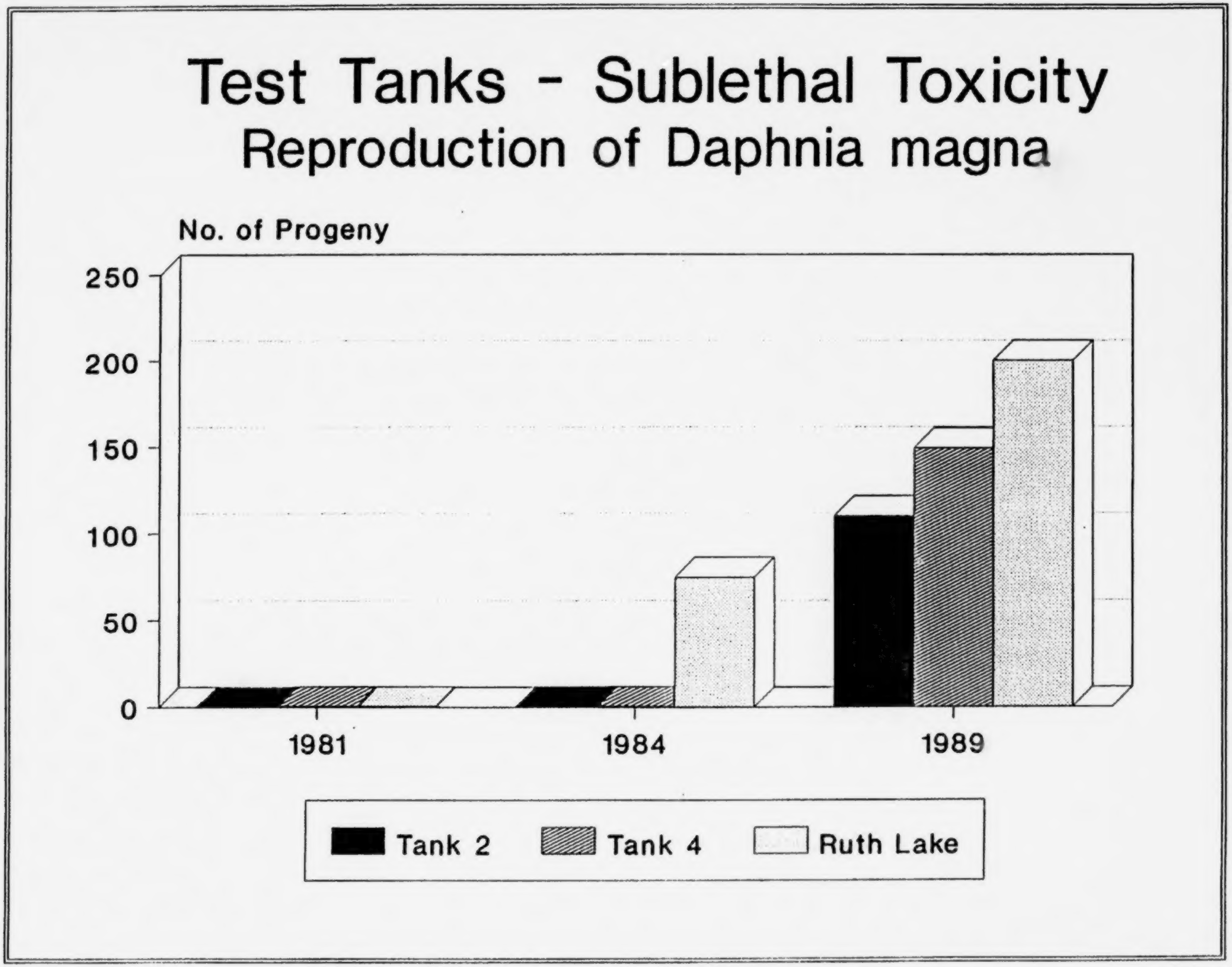

Figure 3. Sublethal toxicity in test tank water using reproduction rates of *D. magna*.

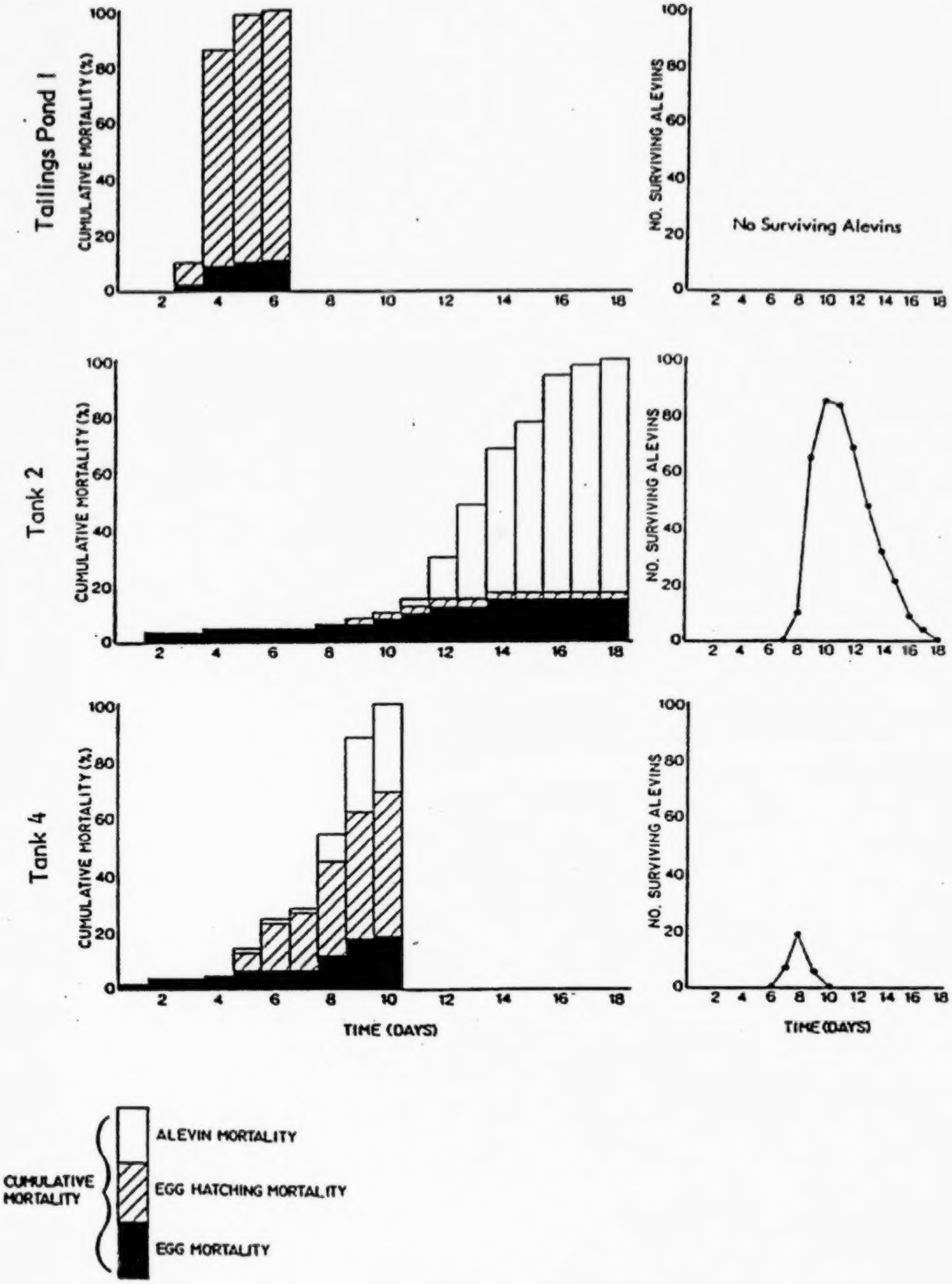

Figure 4. Chronic toxicity in test tanks water using rainbow trout egg hatchability and alevin survival.

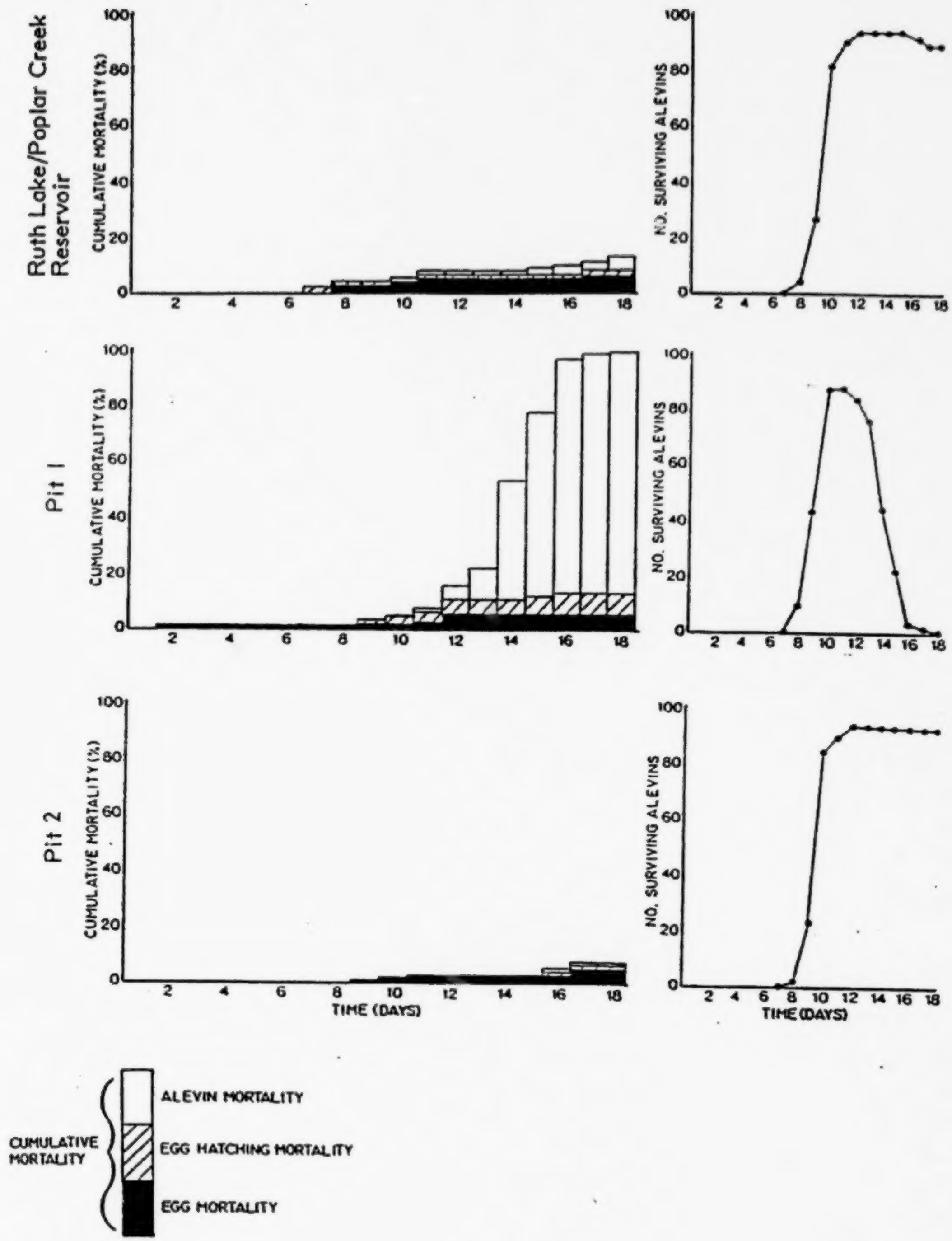

Figure 5. Chronic toxicity data in the test pits using rainbow trout egg hatchability and alevin survival.

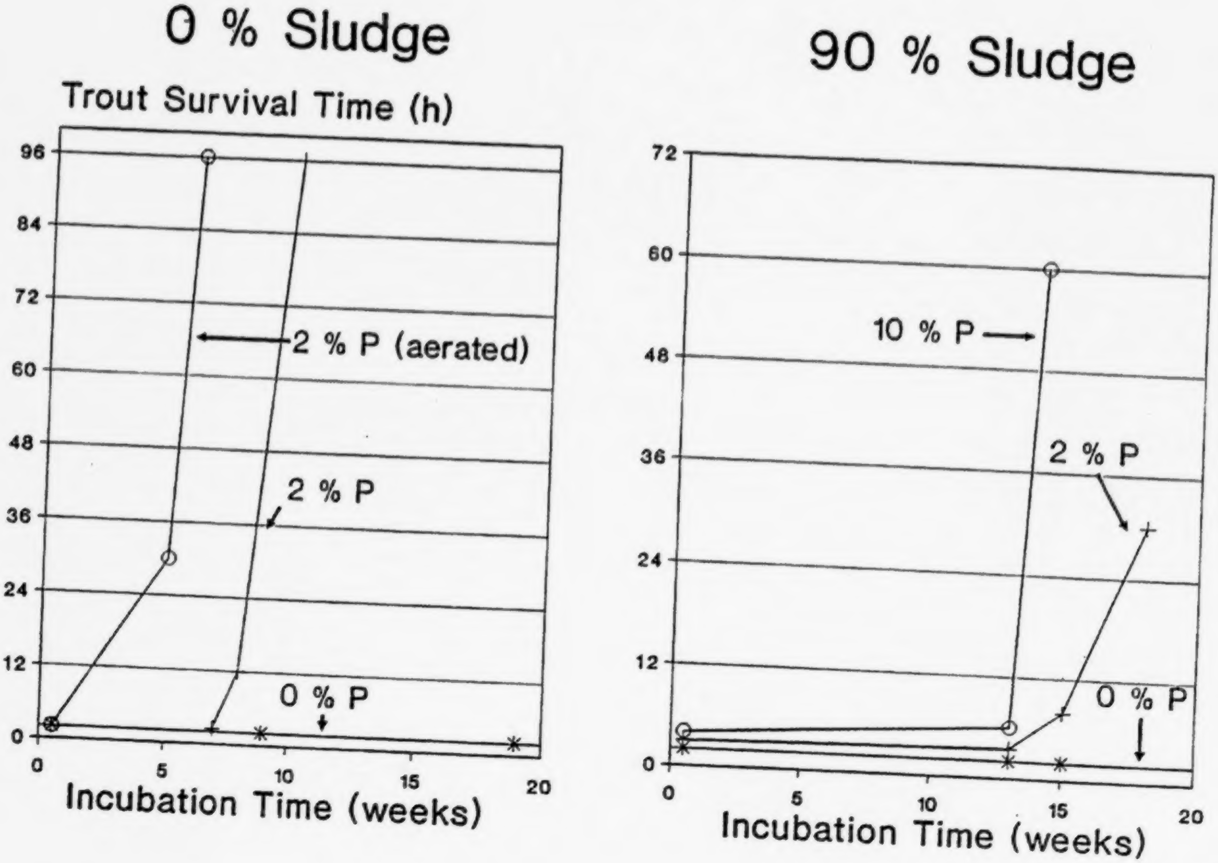

Figure 6. Acute toxicity of tailings pond top water with and without an underlying layer of sludge and under varying concentrations of phosphorus (P).

ROLE OF MICROTOX IN THE DEVELOPMENT OF RECLAMATION STRATEGIES FOR OIL SANDS TAILINGS. **H.W. Hunter, M. MacKinnon, H. Boerger, Syncrude Canada Ltd., P.O. Bag 4009, Fort McMurray, AB., Canada, T9H 3L1, (403-790-5548).**

Since start-up in 1978 Syncrude Canada Ltd., near Fort McMurray, Alberta, has produced about 220 x 10^6 m^3 of fluid wastes (water and solids) during the production of synthetic crude oil. These materials, stored in a 11.5 km^2 tailings pond consist of a low solids surface zone and a high solids sludge zone. The surface water is acutely toxic with a microtox LC_{50} value of 25% to 40%. The sludge zone, containing 25% to 35% solids, has a microtox LC_{50} value of 25% to 40% while the low solids surface water can be reused in the plant operations effective strategies for reclamation of the high solids sludge is more challenging. The current strategy for reclamation of the tailings pond sludge was devised as a result of bench and field scale experiments in which water quality and detoxification rates were monitored through chemical testing and the use of Microtox for toxicity measurement.

OIL SANDS CLAY FINES

Can they be reclaimed as productive, self-sustaining wetlands?

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ABSTRACT

The Clark hot water process currently used for extracting bitumen from the Athabasca oil sands results in large volumes of clay fines containing small amounts of residual bitumen. One possible way of dealing with these fines is to deposit them in abandoned mine pits and cover them with a layer of natural water. Current field trials in a series of 2,000 m^3 pits indicate that a diverse and productive aquatic community can be maintained in the water above these fines. This is due to the properties of the fines and the various processes operating at the fines-water interface. Such water-covered fines would provide suitable habitat for waterfowl.

INTRODUCTION

Syncrude and Suncor, the world's only two commercial oil sands plants, use the Clark caustic-hot water process to extract bitumen from the Athabasca oil sands deposits near Fort McMurray, Alberta, Canada. Processing of a tonne of oil sand results in approximately 0.15 m^3 of mature fines consisting of 30% clay (mainly kaolinite and illite) and a small amount (<1%) of residual bitumen. Currently the two oil sands plants are storing some 200 million m^3 of fines in various tailings ponds and the volume is increasing at about 30 million m^3 per year.

Numerous studies are under way to develop reclamation strategies for these fines. Many of the studies deal with methods for dewatering the fines (i.e. freeze-thaw, evaporation, evapotranspiration, flocculation with chemicals), or else mixing the fines with sand or overburden so as to allow them to be reclaimed as a dry landscape. Another reclamation option, under investigation by Syncrude since 1986 is to deposit the fines in abandoned mine pits and cover them with a layer of natural water.

Because of the rheological properties of these fines and the large density differences between the two layers, the amount of resuspension of the fines into the overlying water column is not considered sufficient to be detrimental to the maintenance of a stable, productive ecosystem in such fines-bottom lakes. Capping fines with water is analogous to land reclamation where barren overburden or tailings sand is capped with a sufficient layer of productive soils to establish a self-sustaining terrestrial community.

Though much work remains to be done, research to date continues to support the concept of capping fines with a water layer. In its 1988 Development and Reclamation Plan, Syncrude proposed to pump mature fines from the tailings pond into the mined-out pit. Pumping is expected to continue for approximately 20 years. As part of the final reclaimed mine area there will be two fines-bottom lakes having surface dimensions of 3 km x 3 km and containing 60 m of fines capped by 5 m of water (Figure 1). The capping water would be taken from the Beaver Creek Reservoir and is expected to evolve into an aquatic system with a biological productivity and diversity (including fish, waterfowl and furbearers) similar to other natural waterbodies in the region.

This paper reviews the results of the various studies carried out so far and discusses a potential end use for such fines-bottomed lakes.

Wind Generated Mixing

Resuspension of fines by wind-generated turbulence was examined in a number of flume tests. These have shown that the threshold bed velocity (U_t) for resuspension of fines is 0.04 m/s. Orbital velocities produced by waves were found to be much more effective in disturbing the fines than linear velocities resulting from currents. At constant orbital velocities of 0.06 m/s the fines were being suspended at a rate of 1 $kg/hr/m^2$, resulting in concentrations of solids of 50 - 100 mg/l within a meter of the fines-water interface.

For a fetch (F) of 4 km and a surface wind speed (U) of 15 m/s, the threshold velocity of 0.04 m/s would occur at a depth of 4.75 m (Figure 2). If fetch and surface velocity are kept constant, then small changes in depth of the capping layer result in significant changes in bed velocity. As shown in Figure 3, increasing the depth from 4 to 5 m results in a 50% reduction in the bed velocity (6 cm/s to 3 cm/s).

Figure 4 shows the relationship between surface wind speed and the depth of water required to obtain the threshold bed velocity of 0.04 m/s. This figure also shows the frequency of storm events with various wind speeds. For a capping layer of 5 m, a storm with sufficient strength to stir up the fines would occur only once every 20 years. If the depth of the capping layer is increased to 5.5 m, it would require a 100-year storm event to disturb the fines.

Release of Interstitial water during sludge consolidation

The fines that will be transferred from the tailings pond into the mined-out pit will have a solids content of 25 - 30% (w/w) and contain less than 100 mg/l bitumen. Additional release of water from the sludge will be very slow and will have a composition similar to that shown in Table 1 (Sludge T_0).

The potential impact of fines interstitial water on the capping layer was investigated using a "worst case" test. Fines with a 30% solids content were placed in a series of plexiglass columns (0.3 m x 0.3 m x 2.5 m) and capped with clean water. The ratio of water to fines was 2:3. In order to increase the rate of fines consolidation and release of interstitial water, a small amount of vibration was applied to each column with an aquarium air pump which was also used to aerate the surface water. The quality of the overlying water was then monitored for 12 months.

As shown in Table 1, the interstitial water released from the fines comprised 29% of the capping water after 12 months. Release of sodium, chloride, sulphate and bicarbonate from the fines led to a 2.4 fold increase in conductivity and a 1.6 fold increase in dissolved solids of the capping water. Dissolved organic carbon did not change but chemical oxygen demand increased 2 - 7 fold. There were also some increase in minor elements (i.e. arsenic, boron), however, the absolute concentration of these is low even in the fines.

Of greatest significance, however, is the fact that the capping water remained non-toxic even though the fines were toxic. Toxicity was measured with the Beckman Microtox Toxicity Meter. In this procedure bioluminescent bacteria are exposed to various concentrations of the test solution for 15 minutes. The presence of toxins results in a reduction in the light output of the bacteria relative to a control culture. Toxicity is expressed in terms of the concentration of the test solution which reduces light output to 50% (EC_{50}) or to 20% (EC_{20}). Fines interstitial water has EC_{50} and EC_{20} values of 30% and 10%, respectively. However, the overlying water had no effect on the light output of the bacteria (EC_{50} and EC_{20} are both 100%).

Construction of Experimental Pits

In light of the positive results from the column study, a decision was made to investigate the concept of fines-bottom lakes in a number of large experimental pits. In June, 1989, seven pits were constructed, each having surface dimensions of 50 m x 10 m and containing 2,000 m^3 of water, water and fines, or only fines (Figures 5 and 6). Pit 1 contains only natural water and serves as a control. Pits 2, 3, 4, and 6 each contain 1,000 m^3 of fines (depth 4 m) and 1,000 m^3 of natural water (depth 3 m).

Table 1: Composition of fines and water used to cap the fines. Changes with time are shown in the composition of the capping water maintained under aerobic conditions by aeration ovr a 12-month period.

	FINES	MILDRED LAKE WATER (CAPPING)*			
VARIABLE	T_0	T_0	$T_{1\ month}$	$T_{9\ months}$	$T_{12\ months}$
pH	8.3	7.9	8.4	8.4	8.5
Conductivity (uS/cm^{-1})	1500	285	340	575	685
Dissolved Oxygen (mg.ℓ$^{-1}$)	<.2	9	8	6	5
Dissolved Solids (mg.ℓ$^{-1}$)	1400	250	250	370	405
Suspended Solids (mg.ℓ$^{-1}$)	30000	29	10	<10	10
Dissolved Organic Carbon (mg C.$^{-1}$)	64	25	16	26	16
Phenols (mg.ℓ$^{-1}$)	0.065	0.003	0.006	0.004	0.003
Cyanide (mg.ℓ$^{-1}$)	0.006	0.001	0.001	0.001	0.001
Chemical Demand Oxygen (mg.ℓ$^{-1}$)	>200	20	48	58	55
Release Water from Sludge** (% of Cap Zone)	-	-	1	20	29
MAJOR IONS					
Cations					
Sodium	450	15	40	106	129
Potassium	11	1.3	2.2	3.4	5.0
Magnesium	4.0	10	9.6	8.8	9.2
Calcium	4.0	38	34	20	16
Anions					
Chloride	130	7.8	13.2	33.6	41.5
Sulphate	4	23	22	22	21
Bicarbonate	940	160	198	280	270
Hardness (mg of $CaCO_3$)	27	135	125	87	78
Ratio Na/Cℓ (meq/meq)	5.3	3.0	4.7	4.9	4.8
Nutrients					
$NO_3^- + NO_2^-$	0.04	0.03	0.02	<0.01	0.31
Ammonia	3.8	0.01	0.20	<.01	0.02
O-PO_4	0.14	0.03	0.02	0.02	0.02
Acute Toxicity					
Microtox					
EC_{50}	30	100	100	100	100
EC_{20}	10	100	100	100	100
Minor Elements (mg.ℓ$^{-1}$)					
As	0.0079	0.0010	0.0018	0.0020	0.0010
B	2.45	0.06	0.16	0.43	-
Cd	<.001	<.001	<.001	0.002	-
Cr	0.016	0.003	0.012	0.007	-
Co	<.001	<.001	0.013	<0.001	-
Cu	0.013	0.012	0.003	0.003	-
Pb	<.002	<.002	0.015	0.015	-
Mo	0.058	<.001	0.017	0.009	-
Ni	0.013	<.001	0.048	<0.001	-
Sr	0.21	0.23	0.24	0.24	-
V	0.023	0.006	0.002	0.001	-

*Ratio of capping layer to fines layer = 2:3.
Capping layer = Mildred Lake Water collected September, 1987.

**Volume of release water from fines as a percent of original volume of capping layer.

Pits 2 and 3 were kept as replicates. Approximately 2 cubic mater of aquatic plants, invertebrates and fish were collected from surrounding water bodies with a seine net and introduced to Pit 4. As well, cattails and other emergent vegetation were dug up from a nearby marsh and replanted in the shallow end of Pit 4. Upon completion, a 1-m broad band of vegetation extended along the entire shallow end of the pit. The objective of these additions was to provide a variety of plants, invertebrates and fish which could be monitored over the ensuing months.

Nutrients were added to Pit 6 in order to boost the overall productivity of the water. This in turn should increase the rate of detritus accumulation at the fines-water interface and accelerate the burial of the fines under a layer of natural sediment. Addition of 500 gm of ammonium phosphate dibasic $(NH_4)_2$ HPO_4 in August and October, 1989 and in May, June, July, and August, 1990 resulted in a 35-fold increase in chlorophyll and primary productivity as determined by the carbon-14 dark and light bottle technique (Table 2).

Table 2: Chlorophyll a (ug/l) and net primary productivity (mg Carbon/m^2/day) for the fines capping experimental pits July 24, 1990. Primary productivity based on a day length of 15 hours and a euphotic zone of 1.75 m (1% of incipient light).

PIT	CHLOROPHYLL a	NET PRIMARY PRODUCTIVITY
1	1	23 ± 0
3	1	20 ± 0
4	3	47 ± 2
5	3	77 ± 1
6	35	845 ± 149

In Pit 5 the 1,000 m^3 of fines were covered with 1,000 m^3 of water from the tailings pond. Although toxic initially (96-hr trout LC_{50} less than 10%), this water will detoxify within 1 - 2 years to a condition suitable for fish growth (Boerger *et al.* 1986, 1987; MacKinnon and Boerger 1987). Since this water is chemically very similar to fines interstitial water, the development of a diverse and productive community in Pit 5 would clearly demonstrate that release of such water from full-sized (3 km x 3 km x 60 m) fines-bottom pits will have no impact on the overlying layer of natural water.

Pit 7 was filled with 2,000 m^3 of fines. The objective here is to further investigate the rate of release of interstitial water from the fines. The larger volume of fines in this pit compared to Pits 2 - 6 will also allow us to assess if any of the fines processes observed in the other pits are dependent on the volume or depth of the fines. This may have some bearing when the results from the pits are extrapolated to full-sized fines-bottom lakes.

Toxicological Analyses

At no time during the last 12 months did the water in Pits 2, 3, 4, and 6 show any signs of acute toxicity based on the Beckman Microtox Test and the standard 96-hour trout test. Lack of acute toxicity does of course not mean that there are no sublethal or chronic effects. To test this possibility, the waters were tested with three different chronic tests in September, 1989 and January, 1990.

In the *Daphnia* life cycle test, these small crustaceans were kept in water from the various pits and their survival and reproduction monitored for seven days. In all eight tests survival was 100% and the number of young produced was not significantly different from that of control animals.

In a second type of chronic test, algae were grown in the water from Pits 2, 3, 4, and 6. In the September tests growth in water from three of the fines-bottom pits was less than in water from the control pit. However, in the January tests, growth in water from the fines-bottom pits was 10 times better than in the water from the control pit.

In a final chronic test, trout eggs were kept in water from the various pits and their development followed for 19 days. Both the hatching of the eggs and the growth of the young alevins are very sensitive to the presence of pollutants. But, as with the other two tests, the water from the fines-bottom pits showed no indication of any chronic toxicity.

Biotic Development of the Pits

Both the fines and the overlying water contain abundant bacteria (10^3 - 10^5 cells/ml, mostly *Bacillus* and *Pseudomonas* sp.), with some indication of higher numbers at the fines-water interface. Based on uptake studies with C-14 labelled glutamate, the bacteria are also metabolically very active. Previous studies (Foght *et al.* 1985) showed that the bacteria associated with tailings are actively decomposing hydrocarbons.

Carbon-14 dark and light bottle measurements made in July, 1990, have shown that Pit 3, which contains fines capped with natural water, to be as productive as Pit 1, the control (Table 2). Addition of biota to Pit 4 has doubled its productivity, while addition of nutrients to Pit 6 has increased productivity 40-fold. Of particular interest, is that Pit 5, which is capped with tailings pond water, has a productivity 4 times higher than Pit 3 which is capped with natural water.

The abundance of algae floating in the water (phytoplankton) has increased steadily in the fines-bottom pits from the time that they were filled (Table 3). However, they did not exhibit a spring bloom as occurred in the control pit. Aquatic macrophytes (*Sparganum, Typha*) are growing in the shallow areas of all fines-bottom pits.

The pits are presently inhabited by a large number of aquatic invertebrates, especially insects. Emergence of the adults is being measured with box-like emergence traps floating on the water surface. As is true for the hatching of fish eggs, emergence of the aerial adult from the aquatic pupal or nymphal stage is very sensitive to the presence of any pollutants. Nevertheless, emergence of insects from the fine-bottom pits is so far not significantly different from that in the control pit.

Table 3: Abundance (cells x $10^3/\ell$) of phytoplankton in experimental pits with and without clay fines.

DATE	NO FINES	FINES
August 17, 1989	177	23
October 15, 1989	116	108
June 4, 1990	180,216	1,154
June 23, 1990	511,595	12,125
July 9, 1990	26,313	12,983

All the fines-bottom pits are inhabited by fish, primarily sticklebacks, fathead minnows and lake chub. These were quite likely introduced during the pumping of the natural water into the pits. An initial survey completed in mid-August, 1990 showed that the size of the fish is not significantly different from that in the control pit and in other surrounding waterbodies, and that reproduction is occurring in all fines-bottom pits. Mr. Allen Verbeek, Department of Zoology, University of Alberta, is currently examining the survival, growth, reproduction and behaviour of these fish as part of a Master's Thesis.

Future Developments

The above results indicate that a diverse and viable aquatic community of plants, invertebrates, and fish will develop in fines-bottom pits. At the current rate of development, it is expected the biota in the pits will be similar to that in surrounding waterbodies within five years. Furthermore, with each passing year the fines will be covered by a thicker and thicker layer of natural sediment resulting from the death of plants and animals and the influx of sand, silt, and clay.

As a final test, it is planned to introduce pike, walleye, and perch as well as waterfowl into the fines-bottom pits. After a period of time the meat of the fish and waterfowl will be tested for tainting. If tainting is shown to be absent, fines-bottomed lakes could be considered suitable for development as waterfowl nesting and staging areas. Development of waterfowl habitat is currently being actively pursued by government agencies in both Canada and United States under the auspices of the North American Waterfowl Management Plan.

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Foght, J.M., P.M. Fedorak, D.W.S. Westlake and H. Boerger. 1985. Microbial content and metabolic activities in the Syncrude tailings pond. AOSTRA J. Res. 1 (3): 139 - 146.

MacKinnon, M. and H. Boerger. 1987. Description of two treatment methods for detoxifying oil sands tailings pond water. Water Poll. Res. J. Can. 21 (4): 496 - 512.

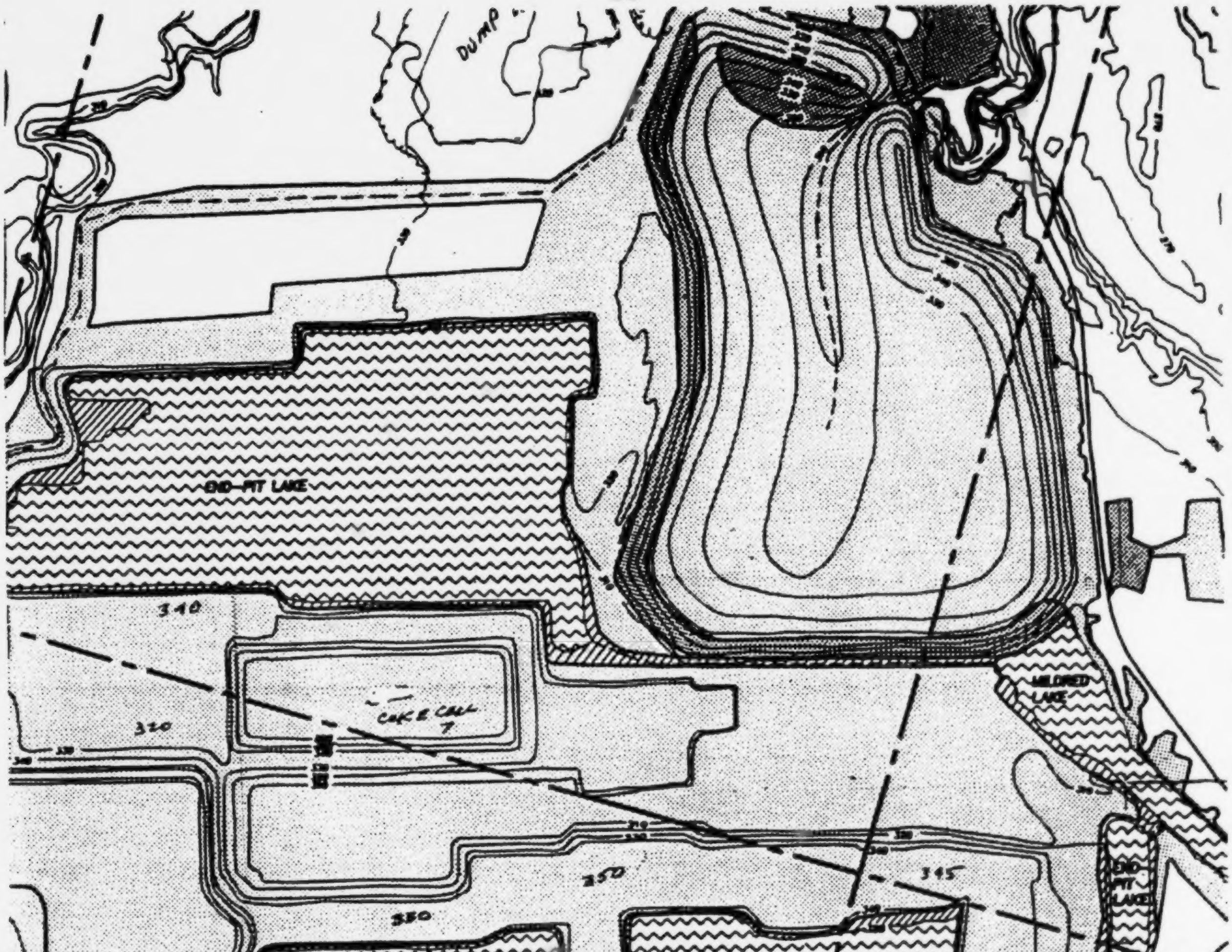
DUMP
END-PIT LAKE
340
320
CAKE CELL 7
350
345
330
MILDRED LAKE
END PIT LAKE
400
SLUDGE BOTTOM LAKE
SLUDGE BOTTOM LAKE
315
370
360
BEAVER LAKE
HIGHWAY 63

Figure 1: Proposed configuration of fines-bottom lakes as described in Syncrude's 1988 Development and Reclamation Plan.

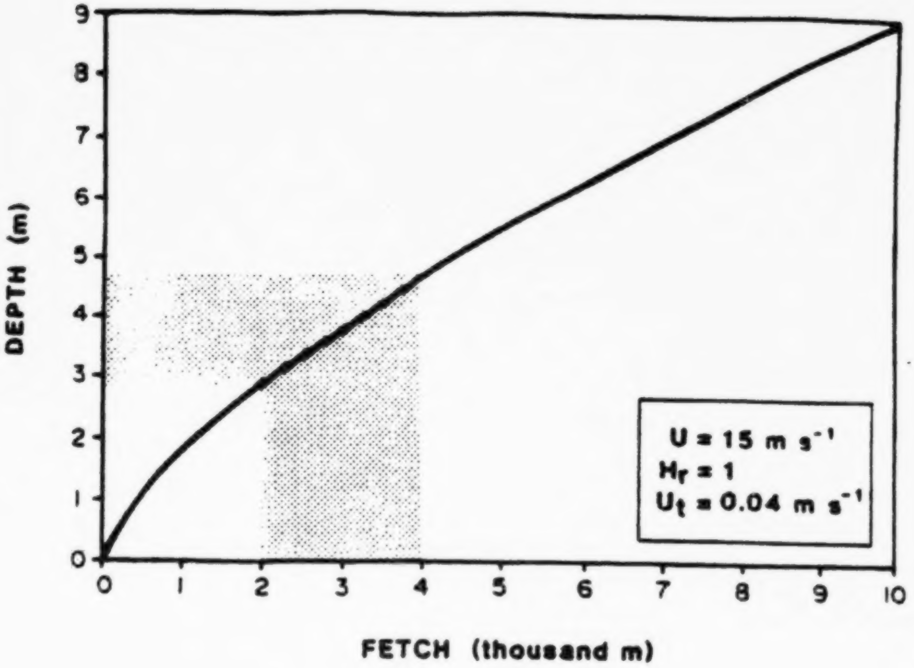

Figure 2: Relationship between fetch and the depth of water at which the wave-induced velocity at the fines interface exceeds the threshold bed velocity (U_t) of 0.04 m/s. Model assumes a surface wind speed (U) of 15 m/s lasting for 4 hours and a wave height ratio (H_r) of 1.

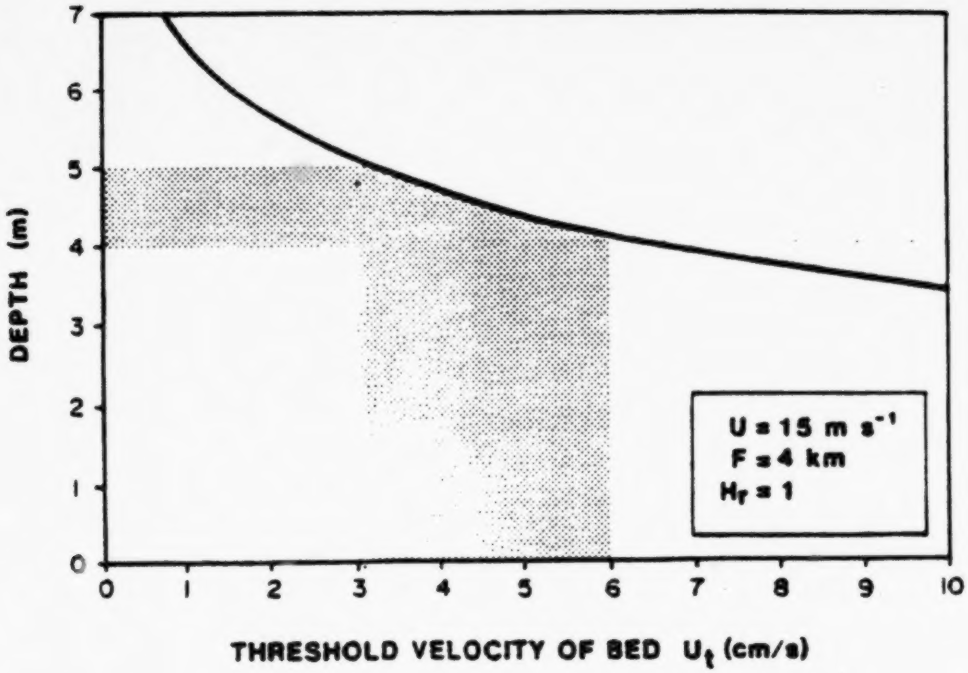

Figure 3: Relationship between threshold bed velocity (U_t) and depth of water for an average surface wind velocity (U = 15 m/s) for a storm of 4 hours duration over a fetch (F) of 4 km. The wave ratio (H_r) of 1 is the ratio of the design wave height to the significant wave height (average wave height if largest one third of waves).

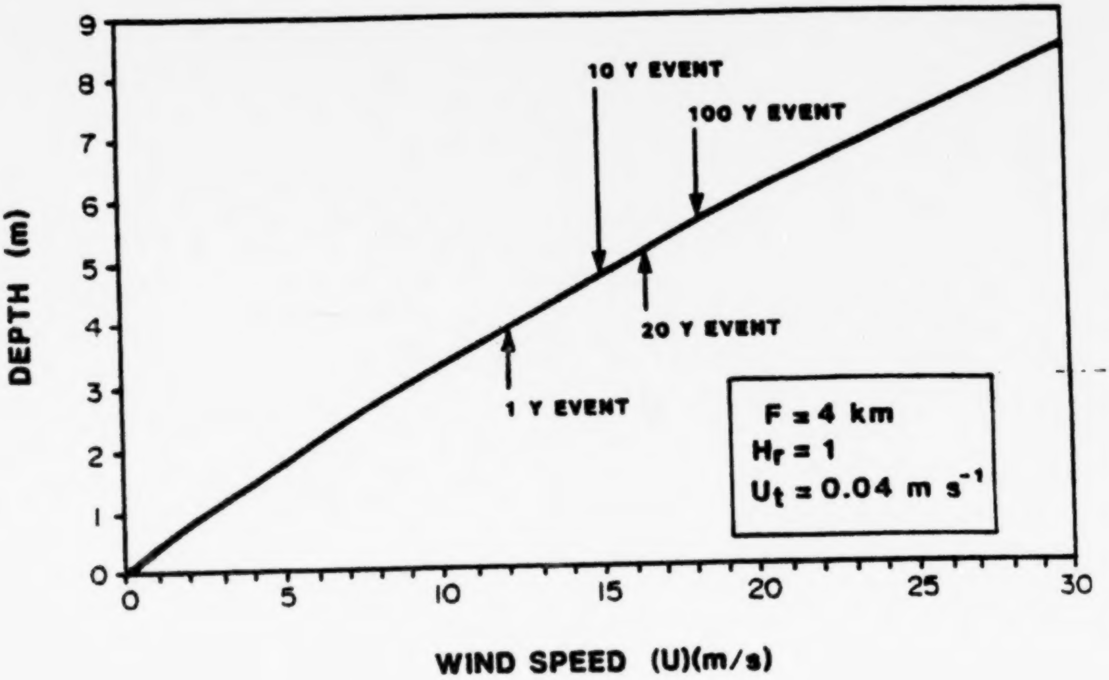

Figure 4: Relationship between surface wind speed (U) and water depth (m) of capping layer required to prevent the bed velocity at the fines interface from exceeding the threshold bed velocity (U_t) of 0.04 m.s^{-1}. Model assumes fetch (F) = 4 km, a wave height ratio (H_r) of 1 and storm duration of 4 hours. Shown on the plot are the positions of the 1 in 1, 10, 20, and 100-year storm events.

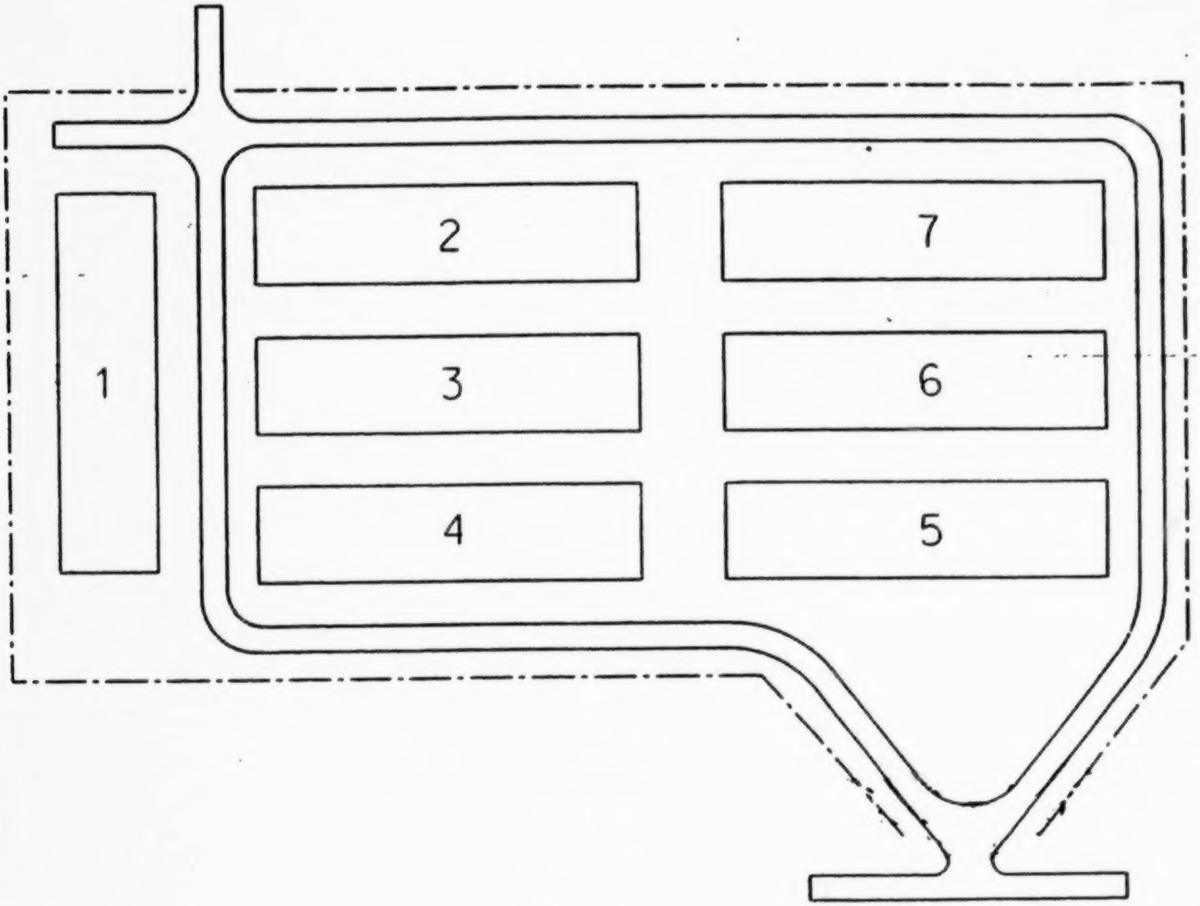

Figure 5: Layout of the fines capping demonstration area. Pit 1 = only natural water (control), Pit 2, 3, 4, and 6 = fines capped with natural water, Pit 5 = fines capped with tailings pond water, and Pit 7 = only fines.

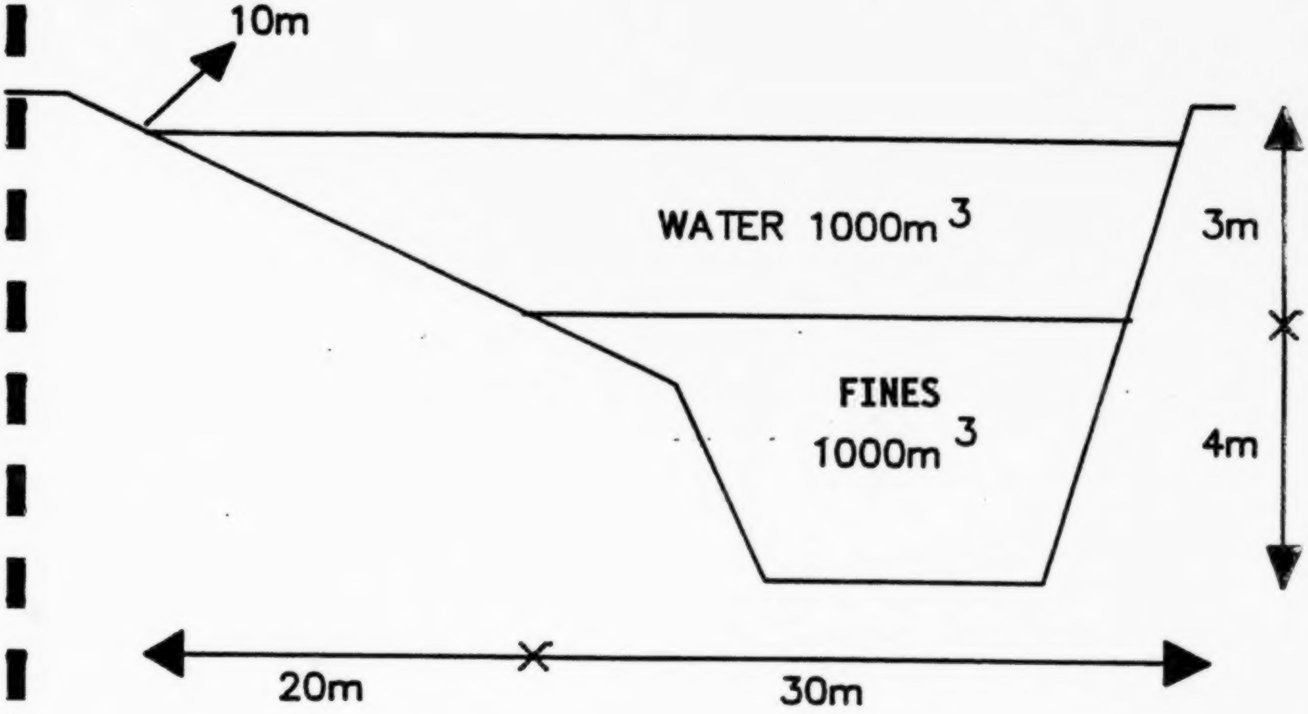
10m
WATER 1000m 3
FINES
1000m 3
3m
4m
20m
30m

Figure 6: Depth profile of Pits 2 - 6. Pits 1 and 7 have same bottom contours but contain only water (Pit 1) or only clay fines (Pit 7).

USE OF ARTIFICIAL SUBSTRATES TO DOCUMENT THE RECOVERY OF SITES IMPACTED BY OIL REFINERY EFFLUENT WATERS.

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ABSTRACT

Benthic surveys using artificial substrates were conducted on the Peace River during 1980 and 1982 near the Petro-Canada oil refinery at Taylor, B.C. The surveys documented a decline in macroinvertebrate abundance (1980:$p<0.001$), richness (1980, 1982: $p<0.001$) and alterations of the taxonomic composition downstream of the effluent discharge. Changes in the invertebrate communities corresponded to elevations in total phenols, ammonia, oil and grease, and temperature. Benthic surveys conducted in 1985 and 1988, after the installation of an activated sludge/biological oxidation wastewater treatment system, found few differences between the invertebrate communities colonizing artificial substrates placed above and below the effluent outfall. By 1988, there were no significant differences in total abundance ($p=0.572$) and taxonomic richness ($p=0.971$) among sites sampled above and below the effluent outfall. The taxonomic composition of benthic communities sampled below the outfall resembled the communities sampled at the upstream reference sites. These data were substantiated by the results of the effluent bioassays and instream water quality analysis. After improvement of the treatment system, rainbow trout 96-h LC50 values exceeded 100% effluent. Chronic 21-d bioassay tests did not indicate any negative effects of effluent exposure on the life cycle of *Daphnia magna*. There were no instream chemical parameters elevated below the outfall. The artificial substrates were an effective method of collecting benthic invertebrates and successfully documented the changes in water quality.

INTRODUCTION

Monitoring changes in benthic community structure has been used extensively to assess environmental impacts of aquatic systems (Davis and Lathrop, 1989). Benthic monitoring programs are commonly conducted by industries that utilize aquatic systems as receiving waters for plant effluent. Benthos may be collected directly from the natural substrate, or indirectly through the colonization of artificial substrates. Typically, sampling from natural substrates is preferred unless sampling conditions make it difficult to do so effectively, e.g. steep slope, hard substrates, large site heterogeneity (Rosenburg and Resh, 1982). At such times, artificial substrates may be used to circumvent these problems. In addition, data from the Lesser Slave River (Munkittrick et al., 1990) indicate that communities collected from artificial substrates are more a function of water quality than site specific sediment quality. As such, artificial substrates should be useful for identifying changes in water quality, and in particular changes in water quality of environments receiving industrial effluents. However, despite these advantages, there are still few examples of the effectiveness of artificial substrates for documenting impacts on benthic communities. The objective of this paper is to describe an example of a monitoring program that was successful in documenting a change in water quality using artificial substrates.

Petro-Canada Inc. operates an oil refinery at Taylor, B.C. on the Peace River. As part of their licence to operate, Petro-Canada Inc. has been required to conduct an operational monitoring study. This program includes benthos monitoring and an assessment of effluent toxicity and instream water quality. An integral part of the benthic monitoring studies conducted by Petro-Canada was the use of artificial substrates to sample benthic communities of the Peace River. Artificial substrates were useful for standardizing substrate conditions among sampling sites and made it possible to sample near the effluent outfall (Cross and Nix, 1986). Standardization of substrate characteristics was important to minimize among site variation which was expected to confound, or obscure, among site comparisons of benthic community structure.

Operational monitoring studies conducted in 1980 and 1982 indicated that the Petro-Canada effluent was impacting the benthic community downstream of the effluent outfall (Noton 1981, 1982). In response to these results, and because the refinery had planned to expand operations by 25%, Petro-Canada installed an activated sludge/biological oxidation wastewater treatment facility in 1983. The effluent quality was expected to improve substantially, decreasing the impact on the receiving environment. Consequently, it was predicted that the benthic communities downstream of the Petro-Canada effluent outfall would, in time, recover.

Benthic monitoring programs were continued in 1985 and 1988 (Cross and Nix, 1986; Nix et al., 1989). Artificial substrates were again utilized to sample the benthic communities upstream and downstream of the refinery effluent outfall. This provided an opportunity to examine how effective artificial substrates were in monitoring changes in benthic community structure in response to improved effluent quality and toxicity. This paper reviews the data collected before and after the installation of the waste water treatment system. The objective was to assesses the effectiveness of artificial substrates to collect benthic invertebrates for the purpose of monitoring spatial and temporal changes in water quality.

MATERIAL AND METHODS

Studies conducted by Noton (1981, 1982), Cross and Nix (1986) and Nix et al. (1989) provided data on benthos, instream water quality and effluent toxicity. For detailed methodology, refer to the above reports. For most years, more than one sampling trip was conducted in the year, typically during the spring and fall. For the purposes of this review, only data collected in the fall were considered. The fall season is usually characterized by a greater abundance of benthic organisms, larger proportion of immature individuals, and increased sensitivity to effluent at low flow and higher water temperatures (Cairns and Dickson, 1971).

During the previous studies, benthos and water samples were collected from sites located upstream and downstream of the Petro-Canada outfall (Figure 1). The number and location of sampling sites varied from study to study, however, the basic design consisted of sites located upstream and downstream of the outfall. River water for chemical analyses was collected immediately below the river surface at each sampling site. Artificial substrates consisted of rock-filled baskets and were suspended in the water column from styrofoam-filled floats anchored in approximately 2.0 m of water. Substrates were left in the river for approximately six weeks for colonization.

Bioassay testing had been conducted to identify possible toxic effects of the Petro-Canada final effluent during the 1986 and 1988 studies. Acute toxicity was assessed using a static 96 h LC50 bioassay with rainbow trout. Chronic/sublethal effects on survivorship, reproduction and growth were examined using *Daphnia magna* life cycle bioassays.

For the purpose of this review, independent statistical analyses were conducted on the data collected during each of the previous studies. The independent analyses were necessary to standardize the statistical methodology and facilitated comparisons among years. The benthic macroinvertebrate data were summarized by calculating the mean abundance (number of animals per substrate basket), taxonomic richness (number of taxa per basket) and the taxonomic composition of each community. Taxonomic composition was examined by calculating the relative abundance (%) of the major taxonomic groups at each sampling site. The differences in total abundance and taxonomic richness among sites were tested using analysis of variance (ANOVA). Linear orthogonal contrasts (Sokal and Rohlf, 1981) were used to examine whether differences in benthic community structure were greatest between upstream and downstream sites. Independent contrasts were also used to examine possible downstream gradients in community structure among sites located below the outfall. Prior to running an ANOVA, benthic data were examined to determine whether the data were normally distributed. Non-normal data were transformed using an appropriated (logarithmic) transformation to satisfy the assumption of normality in parametric statistics.

Cluster analysis was used to determine if there were differences between the benthos communities collected from upstream and downstream sites. Euclidean distance was used as a measure to describe the similarities (or relative distance) in benthic community structure found between any two of the surveyed sampling sites. Using this information, cluster analysis was performed on the derived matrix of Euclidean distance values. Average linkage clustering analysis was employed, using the Euclidean distance data, to elucidate groupings of sites with similar community composition.

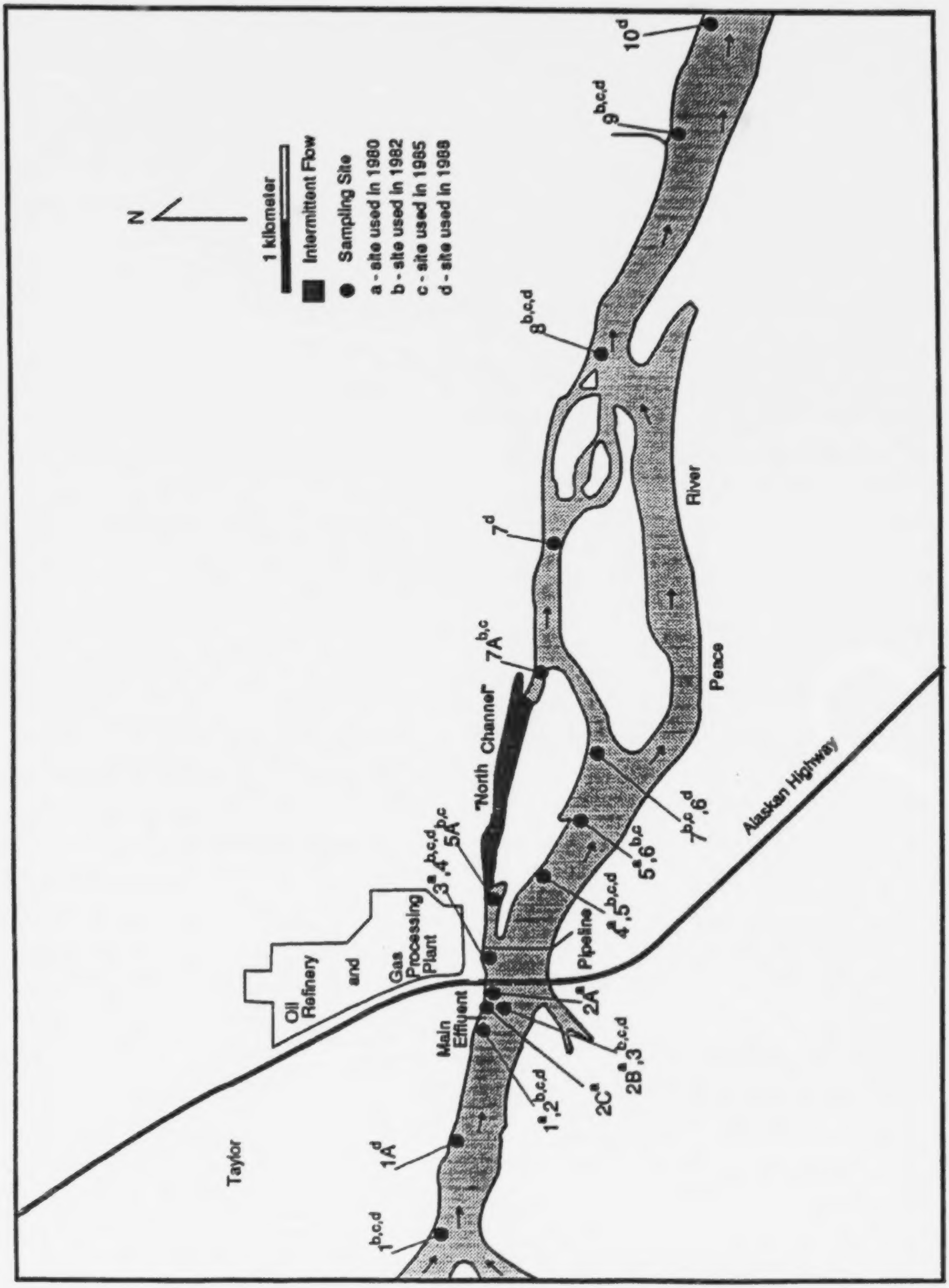

1 kilometer
Intermittent Flow
Sampling Site
a - site used in 1980
b - site used in 1982
c - site used in 1985
d - site used in 1988
N
Taylor
Oil Refinery and Gas Processing Plant
Main Effluent
Pipeline
"North Channel"
Alaskan Highway
Peace River
$1^{b,c,d}$
$1A^{d}$
$1^{a},2^{b,c,d}$
$2C^{a}$
$2B^{a},3^{b,c,d}$
$2A^{a}$
$3^{a},4^{b,c,d}$
$5A^{b,c}$
$4^{a},5^{b,c,d}$
$5^{a},6^{b,c}$
$7^{b,c},6^{d}$
$7A^{b,c}$
7^{d}
$8^{b,c,d}$
$9^{b,c,d}$
10^{d}

Figure 1. Various sampling sites on the Peace River used during the 1980, 1982, 1985 and 1988 benthic monitoring studies conducted by Petro-Canada Inc., Taylor, British Columbia.

Water quality of the Peace River downstream of the Petro-Canada outfall was assessed by comparing instream water parameters to the federal guidelines established to protect freshwater aquatic life (CCREM, 1987). An effort was made to document differences in water quality between the upstream and downstream sites. Effluent toxicity data from the latter monitoring programs was also reviewed to assess the success of the wastewater treatment system.

RESULTS

Water Quality and Effluent Toxicity

Prior to the installation of the wastewater treatment facility, levels of ammonia, oil and grease, phenols and temperature were higher directly below the effluent outfall. The elevated levels of these parameters did not persist downstream (Table 1). In 1980, the concentration of phenols measured directly below the outfall exceeded guidelines stipulated to protect freshwater aquatic life (CCREM, 1987). In 1982, the concentration phenols still exceeded the federal guidelines, however, the concentrations were also high at the reference sites. Concentrations of ammonia were all below the guideline of 1.37 mg/L reported for a pH of 8.0 and a water temperature of 10 °C. There are no absolute guidelines for oil and grease and water temperature.

In 1986 and 1988, chemical levels measured above and below the outfall were similar (Tables 2). Ammonia occurred at levels below the detection limits of the analytical technique. Oil and grease could not be detected in 1985. In 1988, oil and grease occurred in measurable amounts, however, concentrations were not markedly higher below the effluent outfall.and was found in concentrations of phenols were higher at the reference sites than below the outfall during the 1986 study. In 1988, phenols were not detected at any of the sampling sites. Temperature remained higher directly below the effluent discharge, however, these temperatures did not persist downstream.

Rainbow trout 96-h LC50 tests conducted in 1985 and 1988 years indicated that the LC50 values exceeded 100% effluent, demonstrating that the effluent was not acutely toxic to trout. Results from the chronic/sublethal 21-d *Daphnia magna* tests did not indicate any negative effects of the effluent on the life cycle of *D. magna* (Table 3). Tests conducted in 1985 found that the effluent enhanced all life cycle traits except survivorship. Survivorship in refinery effluent was similar to the control results. In 1988, the effluent had no effect on survivorship and total number of moults, but enhanced the total number of progeny produced and the age of first brood, relative to the control.

Table 1. **Water quality parameters which were elevated below Petro-Canada's effluent outfall prior to the installation of the wastewater treatment facility (data from Noton 1981, 1982)**

Fall 1980

Parameter	Site						
	1 (R)	2b	2c	2a	3	4	5
Ammonia	0.05	0.12	<0.05	<0.05	<0.05	<0.05	0.05
Phenol	<0.002	0.027	<0.002	<0.002	<0.002	<0.002	<0.002
Oil & Grease	3	9	2	4	6	3	3

Fall 1982

Parameter	Site							
	1 (R)	2 (R)	DT	3	4	6	7	8
Ammonia	0.06	0.06	0.30	0.06	0.07	<0.05	0.10	0.07
Phenol	0.006	0.018	0.024	0.020	0.018	0.014	0.14	0.014
Temperature (°C)	12.5	11.0	14.5	12.0	12.0	12.0	13.0	11.5

Results given in mg/L unless otherwise specified.

R = reference site located above effluent outfall

DT = discharge turbulence

Table 2. **Selected water quality parameters measured below the Petro-Canada outfall after the installation of the wastewater treatment facility (data from Cross and Nix, 1986; Nix et al. 1988)**

Fall 1985

	Site								
Parameter	1 (R)	2 (R)	3	4	5	6	7	8	9
Ammonia	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Phenol	0.002	0.002	<0.002	<0.002	0.002	<0.002	<0.003	<0.002	<0.002
Oil and Grease	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.8	<1.0
Temperature (°C)	8.8	8.9	10.6	9.4	9.3	9.2	9.0	9.1	9.2

Fall, 1988

	Site						
Parameter	1 (R)	2 (R)	3	4	5	7	10
Ammonia	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Phenol	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Oil and Grease	1.3	1.0	<1.0	2.5	1.0	<1.0	<1.0
Temperature (°C)	14.1	14.1	14.7	14.4	14.5	14.5	14.3

Table 3. Summary of the chronic/sublethal *Daphnia magna* toxicity tests conducted on refinery effluent during 1985 and 1988 (from Cross and Nix, 1986 and Nix et al, 1989)

Date	Survivorship	Total # of Progeny	Total # of Moults	Age at First Brood
1985	0	+	+	+
1988	0	+	0	+

0 = no significant difference compared to the control

- = a significant lower effect compared to the control

+ = a significant higher effect compared to the control

Benthic Macroinvertebrates

Values of total abundance were normalized using a $\log_{10}$ transformation. It was not necessary to transform the taxonomic richness data. Linear orthogonal contrasts were used to compare the abundance and richness values found at the sites located above and below the effluent outfall (Table 4). In addition, contrasts were used to examine downstream gradients in abundance and richness among sites located below the outfall (Table 4).

In 1980, the total abundance of benthic macroinvertebrates was significantly higher at the reference sites compared to sites located below the effluent outfall ($p<0.001$). This pattern was not observed in 1982. Total abundance was not significantly different among the sampling sites ($p=0.455$). In 1985, the values for total abundance were still significantly higher at the reference sites upstream of the effluent outfall ($p=0.006$). The abundance of invertebrates did not recover significantly downstream ($p=0.306$). By 1988, there was no significant difference in total abundance between the reference sites and the downstream sites ($p=0.572$), and there was no significant downstream gradient in abundance among sites located below the effluent outfall ($p=0.713$).

In 1980, taxonomic richness was significantly higher at the reference sites than at the sites located downstream of the effluent outfall ($p<0.001$). This pattern was repeated in 1982 ($p<0.001$), however, there was a significant increase in richness at sites located progressively downstream of the effluent outfall ($p=0.016$). In 1985, taxonomic richness was still significantly lower at sites located below the outfall ($p=0.002$). Values for richness increased progressively downstream ($p=0.047$). In 1988, taxonomic richness observed at sites below the effluent outfall was not significantly different from values observed at the upstream reference sites ($p=0.971$). There remained, however, a positive downstream gradient in richness at sites located below the outfall ($p=0.014$).

Taxonomic composition of the benthos communities sampled at each site was also assessed for signs of effluent-related impacts. Figures 2 and 3 depict the taxonomic composition of the benthos communities found at each sampling site during each of the monitoring years. In 1980, there was a substantial shift from a balanced community structure comprised of Ephemeroptera, Plecoptera, Trichoptera and Chironomidae found at the reference site to downstream communities dominated by Oligochaeta and Chironomidae (Figure 2a). In 1982, the community shift continued and Oligochaeta was the singular most dominant taxon downstream of the effluent outfall (Figure 2b). The upstream reference communities remained diverse and even in dominance. Taxonomic groups such as ephemeropterans, plecopterans, trichopterans, chironomids and other miscellaneous taxa gradually reappeared at the sites located furthest downstream of the outfall. The benthos community structure observed in 1985 indicated that the communities downstream of the Petro-Canada outfall

Table 4. Analysis of variance for linear orthogonal contrasts testing for 1) differences in benthic abundance and richness above and below the outfall; and 2) a positive downstream gradient in abundance and richness among sites located below the outfall.

Date	Dependent Variable	Contrast	P
1980[3]	Abundance	ref. vs down[1]	<0.001
	Richness	ref. vs down	<0.001
1982	Abundance	ref. vs down	0.455
		downstream[2]	0.145
	Richness	ref. vs down	0.001
		downstream	0.016
1985	Abundance	ref. vs down	0.006
		downstream	0.306
	Richness	ref. vs down	0.002
		downstream	0.047
1988	Abundance	ref. vs down	0.572
		downstream	0.713
	Richness	ref. vs down	0.971
		downstream	0.014

1 = contrast comparing reference site to sites located below the effluent outfall

2 = contrast investigating a downstream gradient among sites located below the effluent outfall

3 = too few sites (n = 3) to conduct downstream gradient contrast

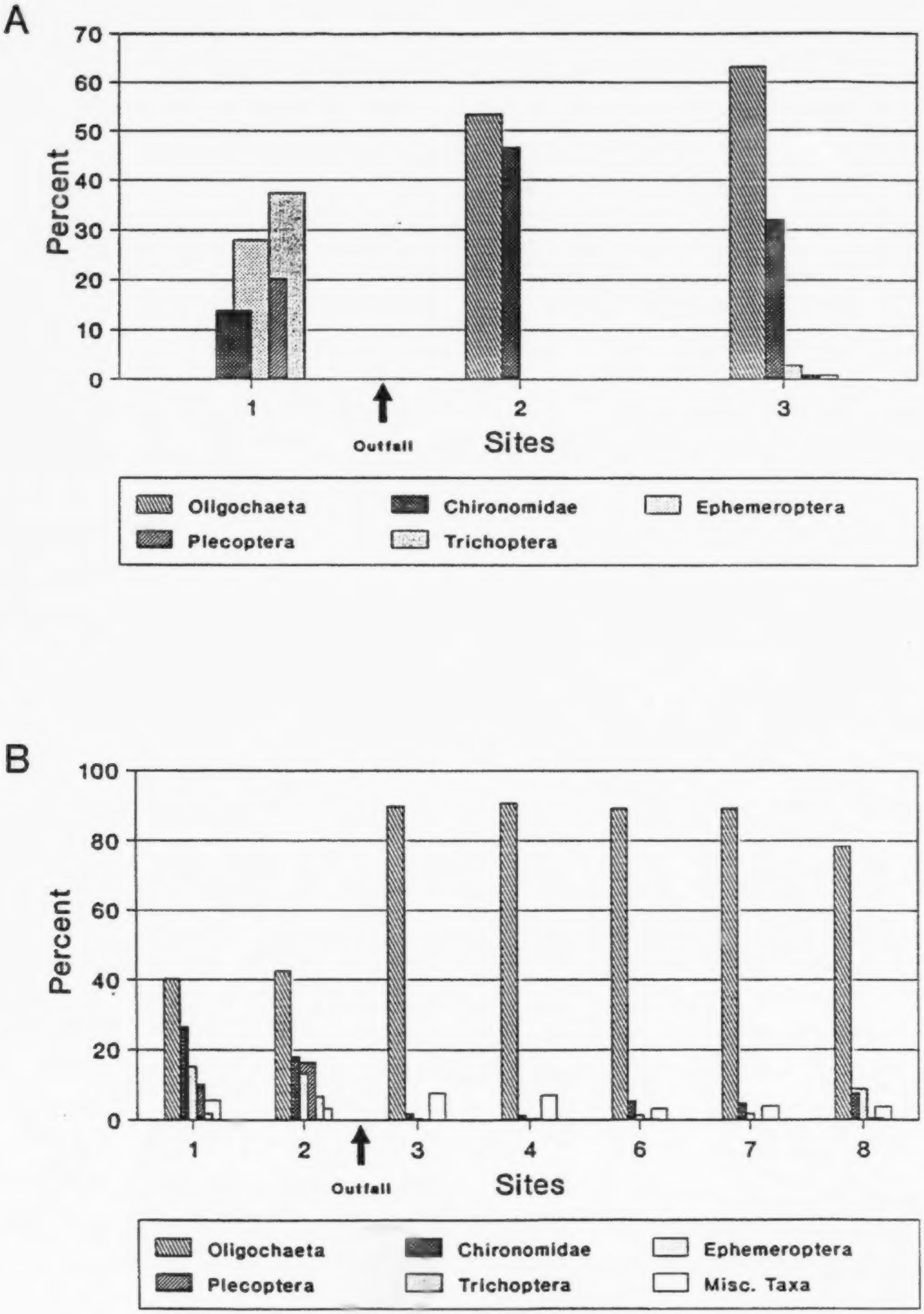


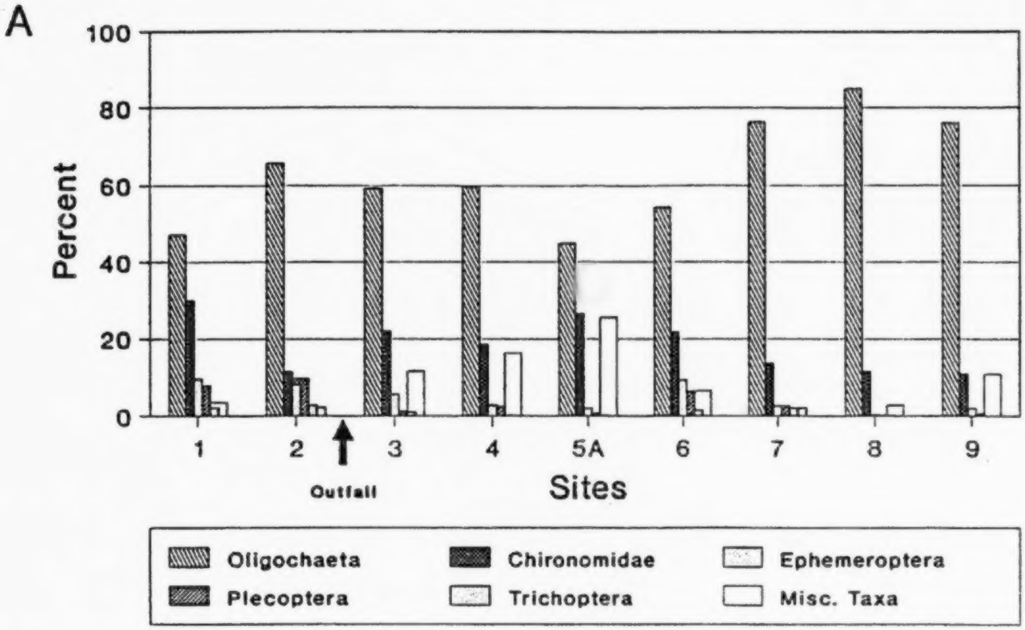
Figure 2. Taxonomic composition of benthic communities sampled above and below the Petro-Canada outfall during the 1980 (A) and 1982 (B) benthic monitoring studies conducted on the Peace River near Taylor, British Columbia.

were not substantially different from the communities observed at the upstream reference sites (Figure 3a). The dominance of Oligochaeta, and to a lesser extent Chironomidae, was still apparent below the outfall, however, other taxa were becoming more dominant than observed in previous years (i.e prior to the wastewater treatment installation). In 1988, the benthos composition downstream of the outfall was not substantially different from that at the reference sites (Figure 3b). The dominance of Oligochaeta below the outfall had disappeared and these organisms were replaced by a community closely resembling that of the reference communities.

Using the site-by-site Euclidean distance matrix, cluster analysis summarized the similarities in benthic community composition among the sampling sites. The results of this analysis are presented in the form of dendograms (Figures 4 and 5). Using the 1980 data, cluster analysis successfully separated the three sampling sites into two distinct groups, or clusters (Figure 4a). The upstream reference site was separated into a group by itself. The second cluster consisted of sites 2 and 3 which were located below the effluent outfall. Using the 1982 data, cluster analysis separated the sites into two clusters corresponding to upstream (sites 1 and 2) and downstream (sites 3-8) locations (Figure 4b). The downstream group was composed of two smaller groups, with site 7 in a group by itself. The dendogram for the 1985 data show that cluster analysis categorized the sites into two clusters (Figure 5a). The first cluster consisted of reference sites 1 and 2, and downstream sites 3 and 4. The second cluster consisted of downstream sites 5A,6,7,8 and 9. The grouping of sampling sites was independent of the location of the effluent outfall. Cluster analysis was moderately successful in separating the 1988 community structure into two clusters (Figure 5b). The first cluster consisted of sites 5,6, and 9. The second cluster consisted of sites 1,1A,2,3,4,7,8 and 10. As in 1985, the clustering of sampling sites was independent of the location of the Petro-Canada effluent outfall.

DISCUSSION

Artificial substrates were an effective method of collecting benthic invertebrates for the purpose of monitoring changes in water quality. Changes in total numbers, taxonomic richness and composition of benthic communities collected from these substrates reflected changes in the water chemistry of the receiving environment. Prior to the installation of the wastewater treatment facility in 1983, the ability of benthic macroinvertebrates to colonize artificial substrates was reduced. During 1980 and 1982, when levels of phenol, ammonia, oil and grease and temperature were elevated, there was a general decrease in abundance and richness associated with the communities sampled below the effluent outfall. With the improvement in effluent quality due to the treatment facility, the quality of the receiving water also improved. This was reflected by the increase in benthic colonization of the artificial substrates.



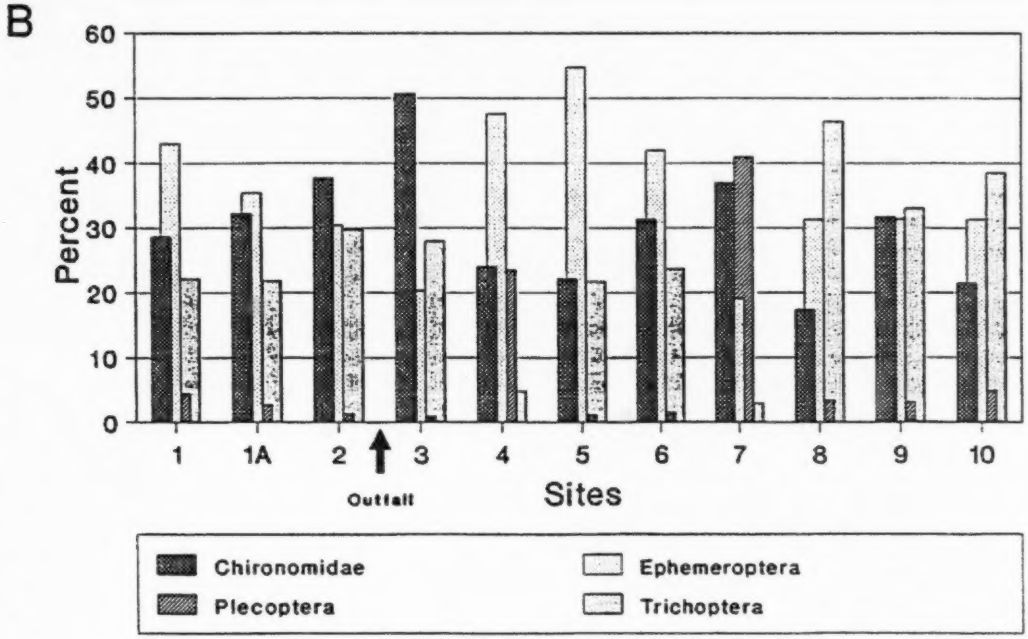


Figure 3. Taxonomic composition of benthic communities sampled sampled above and below the Petro-Canada outfall during the 1985 (A) and 1988 (B) benthic monitoring studies conducted on the Peace River near Taylor, British Columbia.

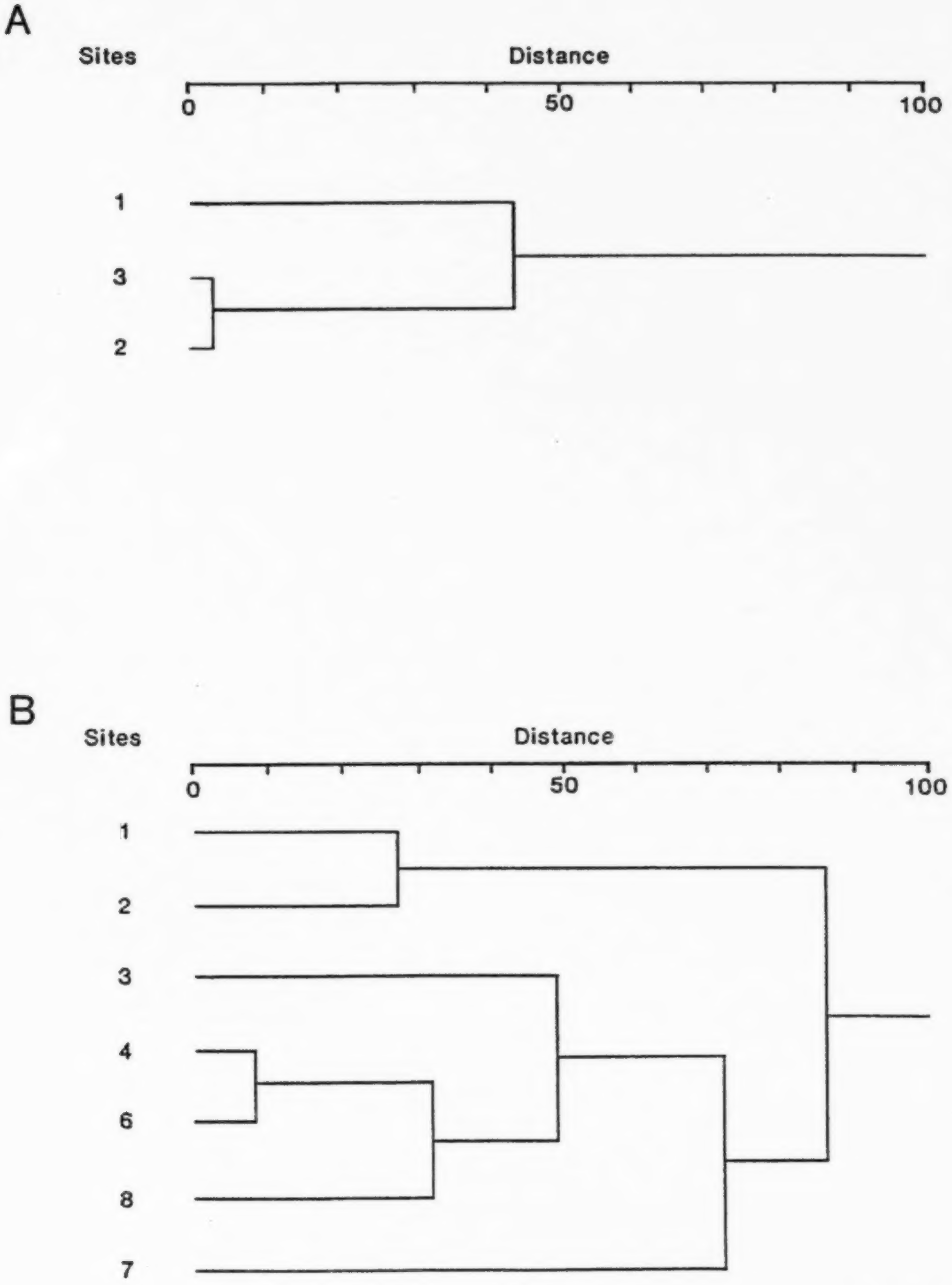
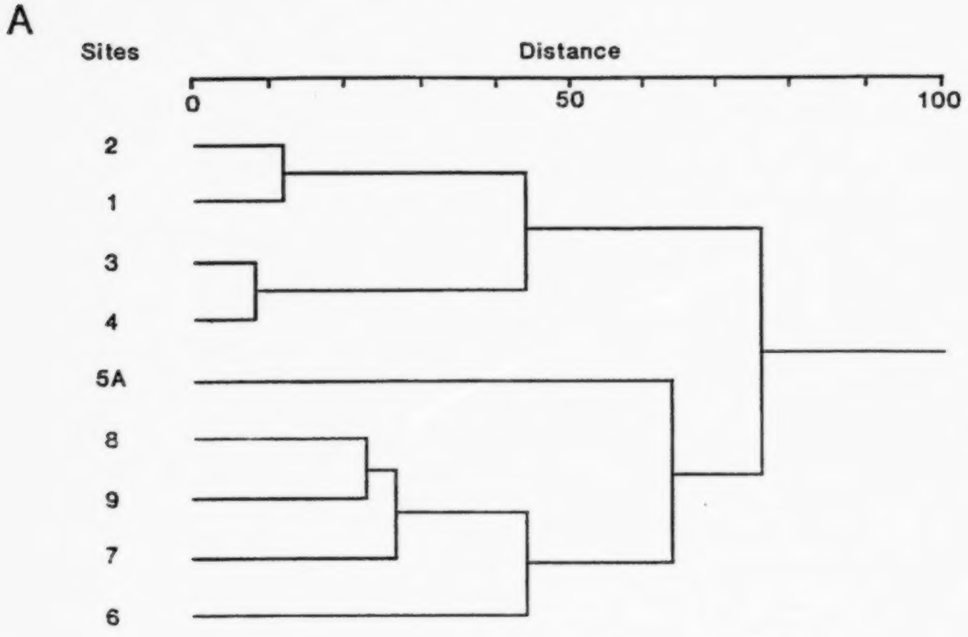


Figure 4. Average linkage clustering of euclidean distance values for sites sampled above and below the Petro-Canada outfall during the 1980 (A) and (B) 1982 benthic monitoring studies conducted on the Peace River near Taylor, British Columbia.



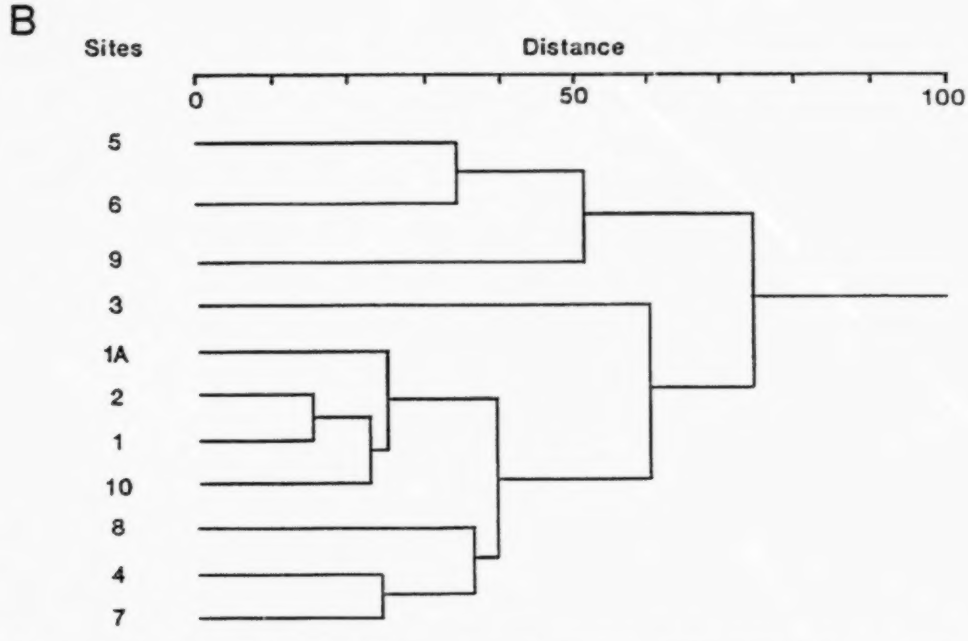


Figure 5. Average linkage clustering of euclidean distance values for sites sampled above and below the Petro-Canada outfall during the 1985 (A) and (B) 1988 benthic monitoring studies conducted on the Peace River near Taylor, British Columbia.

There have been many studies which have assessed the spatial changes in water quality (e.g. below an industrial outfall) using artificial substrates (e.g. Gibbons et al., 1990). However, these studies represent short term impact studies and are vulnerable to unpredictable fluctuations in the aquatic system (Green, 1983). Unfortunately, there are few studies (e.g., Williams, 1980), known to the author, which have used artificial substrates to document long term changes in water quality. The current review, however, indicates that artificial substrates can successfully document changes in instream water quality over time. Consequently, when conditions are not appropriate for the collection of natural substrate samples, artificial substrates are an effective and reliable means to sample benthic invertebrates for the purpose of instream monitoring.

ACKNOWLEDGEMENTS

All data were provided by Petro-Canada Inc.. I would especially like to thank Mr. Ed Scriba, Mr. Francois Roberts, and Mr. Carl Reimer of Petro-Canada. Editorial comments from Mr. Bruce Kilgour, Dr. Mike Paine and Joseph Brador were greatly appreciated. Tables were done by Arlene Booth, Ruth Burr and Janice Linton. Figure 1 was produced by Jean Hays Backman.

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METHODS TO DETERMINE THE EFFECTS OF HERBICIDES ON PRIMARY PRODUCTIVITY IN LOTIC ECOSYSTEMS.

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EXTENDED SUMMARY

Introduction

A major community in all aquatic ecosystems is the Aufwuchs or periphyton which consists of a diverse, ecologically important group of algae and other microbiological organisms growing underwater on natural substrates. As primary producers, the periphyton provide both a habitat and a major energy source for many larger aquatic organisms and thus play a very important role in the structure and function of aquatic ecosystems. In rivers and streams, the periphyton are one of the first groups of biota to be exposed to contaminants from point and non-point sources and by virtue of their immobility, cannot escape the impact of toxic chemicals in their ambient environment.

Most pesticides enter a riverine environment as a concentrated dose in surface runoff or accidental overspray which becomes quickly diluted as the chemical moves downstream. Very few methodologies are available to determine the short- and/or long-term effects of these chemicals on attached algal communities. The **objective** of this paper is to describe several methods presently being tested to determine the in situ impact of pesticides on periphyton in streams.

Artificial Substrates

For the past three growing seasons, the effects of different levels of two herbicides, atrazine and metolachlor, on the growth of attached algal communities in agricultural streams adjacent to land with differing tillage practices have been studied using artificial substrates. The two types of artificial substrates were as follows: a) 15 cm X 15 cm unglazed ceramic tiles standing in wire baskets parallel to the stream flow and b) 0.6 cm acrylic rods standing upright in a weighted stand. Four replicate tiles and rods were sampled on each sampling date following installation for a number of weeks during the growing season. During sampling, a known area of each substrate was scraped into a vial and transported on ice to the laboratory. In the laboratory, samples were homogenized with a blender and subsamples were taken for separate measurements of ash-free dry weight, chlorophyl a, and species diversity over time. Results indicate that the use of artificial substrates provide a uniform, replicable surface area for growth of algae and the collection of samples. In addition, they allow comparisons of sites with different levels of contamination over time.

Portable Bankside Incubator

Experiments were performed using a specially constructed **portable bankside incubator** to study the short-term effects of several agricultural (atrazine, metolachlor) and forestry (hexazinone) herbicides on the primary productivity of attached algal communities in streams. Natural substrates (rocks) with attached algae were incubated in individual 1.5 L cylindrical plexiglass chambers (12 cm diameter, 16 cm high) placed in a clear plexiglass tank. The water in the chambers was kept in circulation by magnetic stir bars fitted with vertical baffles spun by rotating horseshoe magnets chain-driven by a motor and generator. Stream water was circulated through the plexiglass tank with a 12V bilge pump to maintain chambers close to stream temperature. Triplicate chambers were either maintained as controls or dosed with concentrations of pesticides close to those expected in the aquatic environment. Primary production was measured using the light-dark method while monitoring oxygen concentrations using a YSI Model 54 dissolved O_2 metre and expressed as mg $O_2/m^2/hr$. Using this methodology, concentrations of herbicides which cause a significant reduction in primary productivity, albeit on a short-term basis, can be determined.

FACTORS AFFECTING PHOTOSYNTHETIC FLUORESCENCE INDUCTION FROM ALGAE GROWN IN MICROPLATES.

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EXTENDED SUMMARY

A bioassay protocol has been developed to obtain the essential phytotoxicological data on priority in-use pesticides for Canadian water quality guideline derivation. The purpose of this study was to determine the principal physical factors affecting the photosynthetic fluorescence induction response (Kautsky effect) of algae grown in microplates. It was found that levels of relative fluorescence units (R.F.U.) varied heterogeneously in a microplate whose 96 wells were inoculated with a culture of *Selenastrum capricornutum* at constant inoculum conditions for a period of four days. In order to account for this heterogeneity, the following parameters were analysed: effect of evaporation processes during incubation, cell concentration, inoculum volume, probe distance, adjacent well proximity, plate well shape and plate opaqueness. It was found that intraplate variation of R.F.U. levels occurred as a result of media evaporation from peripheral wells. R.F.U. levels were directly proportional to the inoculum cell concentration and to the inoculum cell volume but indirectly proportional to the inoculum distance from the light harvesting probe. The contiguity of the well inocula affects the R.F.U. response as a well with inoculated surrounding wells gave a reduced response than a well with its neighbouring wells empty. The response was greater in round bottom plates as compared to flat bottom plates. Dark plates gave a slightly reduced response when these were compared to translucent plates. The microplate lid and the anti-evaporation plastic bag absorbed more than half the light at and below 400 nm. It is recommended that peripheral wells be included in experimental designs but excluded from data analyses. This bioassay procedure was found to be a rapid, cost-efficient and scientifically sound technique to obtain the necessary information requested for phytotoxicological data evaluation.

MICROBIAL TEST SYSTEMS FOR ECOLOGICAL ASSESSMENT. C.W. Hendricks, US EPA Environmental Research Laboratory, Corvallis, OR 97333; and S. Smith, NSI, U.S. EPA Environmental Research Laboratory, Corvallis, OR 97333 (503-757-4777)

The US EPA's activities of identifying, characterizing, and cleaning up hazardous waste sites are regulated by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) and the Superfund Amendment and Reauthorization Act of 1986 (SARA). Both CERCLA and SARA address the effects of toxicants to terrestrial and aquatic life; consequently, techniques to measure toxicity are not only important to risk analysis, but also pertinent to baseline assessments for biorestoration projects.

Research has been initiated to investigate the utility of standard microbial toxicity tests that could be used as part of a toxicity test battery prior to the design of site remediation and restoration protocols. In this study EC_{50} values for Algal, Microtox, Toxi-chromotest, and Nitrifying bacterial bioassays using 11 organic chemicals and 8 metals were calculated. EC_{50} estimates with Microtox were made with both the recommended NaCl diluent as well as sucrose. The results of this study indicated no one assay was "best" but similar trends were evident.

THE PRINCIPLES AND PROCEDURES OF DEVELOPING A BIOLOGICALLY-BASED TOXICOKINETIC MODEL FOR CHEMICALS IN SALMONIDS.

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Toxicokinetics is the study of the time course for the absorption, distribution, metabolism, and elimination of a chemical in a biological system. Toxicokinetic models generally are divided into two categories: classical and biological. The biologically-based toxicokinetic model (BBTM) differs from the classical model in that it is developed with the actual physiological and biochemical parameters of the organism. The chief advantage of of a BBTM is its greater predictive power. Therefore, the BBTM has been used to reduce uncertainties in the risk assessment process (NRC, 1987; USEPA, 1987) and increase understanding of species differences in chemical toxicities. However, very few BBTMs have been developed in the fish. Previously published BBTMs of fish include only those of methotrexate in stingrays (Zaharko et al., 1972) and phenol red in dogfish shark (Bungay et al., 1976). In this presentation, the principles and procedures involved in the development of a BBTM will be reviewed and illustrated with the recently developed pyrene and oxytetracycline models of salmonids. The potential applications of BBTM in aquatic toxicology also will be disscussed. (Supported by NSERC and B.C. Science Council).

Dombroski, E.C., I.D. Gaudet, and A.A. Qureshi. 1990. Review of the Microtox Methodology for Toxicity Assessment of Solid Matrices. Can. J. Fish. Aquat. Sci.

Introduction

The Microtox[1] assay is designed primarily for use in the analysis of aqueous samples; it cannot test the toxicity of a solid sample directly. The toxicants in solid matrices must be extracted from the solid sample into a suitable solvent so that a portion of the extract may then be subjected to the Microtox assay for toxicity assessment. The procedure used to extract toxicants from a solid sample is critical to the analytical result. Reported methods for preparing aqueous and organic extracts of solid or semi-solid samples (such as soil, sediment, sludge, drilling fluids and wastes) for Microtox testing were found to be extremely variable with little or no standardization across studies. Explicit rationale for choosing one particular procedure over any other reported in the literature were often lacking. As a result, new studies tend to adopt published methods piecemeal, adding their own modifications as required. All of this has made it difficult, if not impossible, to meaningfully compare data and evaluate procedures across studies.

In this review we have attempted to critically evaluate published information describing methods used to apply the Microtox assay to toxicity analysis of solid and semi-solid samples. To this end, more than 150 papers reporting on the use of Microtox and other bioassays were reviewed. Almost a quarter of these dealt specifically with toxicity assessment of solid samples, 20 of which were considered to be representative of the different methods of aqueous and organic extraction currently in use (these are listed in Tables 1 and 2). None of the studies reviewed made any attempt to detect toxicity of highly volatile components of a solid sample and so this was not considered here.

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[1] Microtox is a trademark of Microbics Corporation 2232 Rutherford Road, Carlsbad, CA 92008.

Based on the analysis and evaluation of the methods data compiled, we recommend the development of standardized extraction procedures, which must precede Microtox testing of solid samples, according to the nature and type of sample (i.e. physical attributes) and toxicants present (i.e. chemical composition). Additionally, some recommendations are made to maximize the usefulness of data produced in future studies.

Extraction Procedures and Discussion

Adapting the Microtox assay to the analysis of toxicity testing of solid samples depends upon the delivery of the toxicants to the test organism, which is in an aqueous environment. Various methods have been developed and used to bring the toxicants in contact with the test organisms. The methods reviewed were divided into two major categories based on their use of aqueous or organic solvents to extract toxicants from the sample (Tables 1 and 2, respectively).

Aqueous Extraction

Testing aqueous extracts of solid samples for toxicity provides information on the water soluble fraction of toxicants present. These toxicants can pose a significant problem since, being water soluble, they can be readily bioavailable. Water solubility also introduces the problem of mobility, especially where toxic leachate from sediment or soil has the potential of entering and contaminating surface or groundwater (Chapman and Becker 1986, Matthews and Bulich 1986, Matthews and Hastings 1987, Ross and Henebry 1989, True and Heyward 1990).

Table 1. Procedures for aqueous extraction for Microtox bioassay of solid samples.

Sample Type	Solvent [sample:solvent]	Mix by (Time)	Separate by	Reference
solid	DW [1:5 (w:v)]	shaking (24 h)	settle	Beckman 1982
solid waste, sludge	DDW, aqueous buffer [1:10, 1:16, 1:20]	stirring (4, 24 h)	N/A	Calleja et al. 1986
drilling fluids	N/A	N/A	sequential filtration, mud press	Moynihan et al. 1988
soil	DDW [50 g:400 Ml]	tumbling (22 ± 2 h)	filter (0.45 um, under pressure)	Matthews and Bulich 1986
soil	DDW [100 g:400 mL] / none if sample was >50% water	tumbling (22 ± 2 h)	centrifuge 10 min, 2500 rpm ± filter (0.45 um)	Matthews and Hastings 1987
soil	DDW [25 g:100 mL]	tumbling (22 ± 2 h)	settle, centrifuge 15 min, 3000 rpm	Symons and Sims 1988
sediment, dredges	DDW, seawater [1:4 (v/v)]	aeration (30 min)	centrifuge 30 min, 6500 x g / filter (1.2 um glass wool)	Ankley et al. 1989
sediment	DDW [$4L\cdot Kg^{-1}$]	shaking (48 h)	filter (0.45 um cellulose-acetate)	Athey et al. 1989
sediment	DDW [1:1]	shaking (3 min)	centrifuge 10 min, 5000 rpm	Dutka and Kwan 1988
sediment	N/A (pore water)	N/A	centrifuge 45 min, 5000 x g / filter (1.2 um GFF, 20 psi)	Giesy et al. 1988a & b
sediment	DW	aeration (2 h)	settle, filter (1.2 um glass fibre filter)	Ross and Henebry 1989
sediment	Microtox diluent [30 g:10 mL]	shaking (24 h)	centrifuge 15 min, 9770 x g	Tetra Tech, Inc. and E.V.S. Consulting, Inc 1986
sediment	none (interstitial)	shaking (22 ± 2 h)	centrifuge 20 min, 10,000 x g	True and Heyward 1990
sediment	Microtox diluent [13-26.4 g:10 mL]	shaking (24 h)	centrifuge 15 min, 9770 x g	Williams et al. 1986

DW (distilled water), DDW (distilled, deionized water), GFF (glass fibre filter), N/A (not applicable)

In studies where aqueous extractions were carried out (Table 1), the procedures reported could be reduced to three basic steps.

1) Addition of an aqueous solvent to the solid sample.
2) Mixing to facilitate partitioning of the water-soluble toxicants into the solvent.
3) Separating/Collecting the aqueous phase and subjecting a portion thereof to the biological toxicity assay.

Addition of an aqueous solvent

Distilled, deionized water was generally the solvent of choice (Table 1). Saline solutions such as seawater or Microtox diluent (2% sodium chloride) were also used, for example, when studying toxicity of leachate from marine sediments (Ankley et al. 1989; Tetra Tech, Inc. and EVS Consultants, Inc. 1986; Williams et al. 1986). Calleja et al. (1986) were the only authors found to have utilized aqueous buffers as solvents in order to control the pH of the extraction. No solvent was added at all in several studies where the interstitial water made up more than 50% of a sample (Matthews and Hastings 1987), the aqueous portion of drilling fluid was being tested (Moynihan et al. 1988), or the toxicity of the interstitial water was of specific interest to the investigators (Giesy et al. 1988a and b).

Each of the solvent systems mentioned above has certain advantages and disadvantages. Using distilled water is perhaps the simplest, the only adjustment required is that the final sample be adjusted to 2% sodium chloride in order to protect the osmotic integrity of the test organism. Using seawater as the solvent may have advantages when modelling environmental conditions for marine sediments, but it also introduces some uncertainty into the characteristics of the extraction solvent (e.g. the degree of salinity). It is not always necessary to adjust the osmotic strength of the seawater extract immediately prior to performing the Microtox assay (Ankley et al. 1989). However, appropriate controls should be run concurrently since changes in salinity could affect bacterial luminescence (Ribo and Kaiser 1987). Adjusting the pH of the extraction solvent has the potential of exercising selectivity over the range of toxicants which may be extracted from a solid sample. This approach is limited to some extent because as the pH of the final sample approaches the tolerance limits of the test organism, a rapid decrease in bacterial luminescence is observed (Ribo and Kaiser 1987).

Solid to solvent ratios (w:v) varied widely from 1:1 (Dutka and Kwan 1988) to 1:20 (Calleja et al. 1986). The most common ratio used was approximately 1:4.

Choice of solid to solvent ratio will have a significant effect on the degree of response seen in the assay. If a study is simply qualitative (presence or absence of toxic response), then using less solvent would provide a relatively concentrated extract and therefore a more easily detected response. Alternatively, using more solvent to increase the efficiency of the extraction, while still eliciting a detectable response, would more accurately relate the magnitude of toxic response to a discrete amount of sample (e.g. per g dry weight). Results could then be used in a semi-quantitative manner comparing relative toxicities of different samples or changes in toxicity over time.

Mixing

Mixing was usually conducted by placing samples on a rotary shaker, wrist-action shaker, or a tumbler at a moderate speed. Mixing times varied from 3 minutes (Dutka and Kwan 1988) to 48 hours (Athey et al. 1989). Aeration has also been used for sample mixing (Ankley et al. 1989, Ross and Henebry 1989).

The duration of mixing should be long enough to ensure that equilibrium conditions have been achieved with respect to the types of toxicants present (i.e. their chemical composition) and the physical characteristics of the sample. The chances of contaminating the sample or altering its character through loss of volatile components and enhanced oxidation reactions would weigh against using aeration as a routine method of sample mixing.

Separating/Collecting the aqueous phase

Methods for separating the aqueous phase included simply letting the solids settle out (Beckman 1982); centrifugation (Ankley et al. 1989; Dutka and Kwan 1988; Symons and Sims 1988; Tetra Tech, Inc. and E.V.S. Consultants, Inc. 1986; True and Heyward 1990; Williams et al. 1986); filtration using cellulose filter paper (Matthews and Bulich 1986), cellulose-acetate filter paper (Athey et al. 1989), or glass fibre filters (Ross and Henebry 1989); or a combination of these techniques (Matthews and Hastings 1987, Giesy et al. 1988a and b).

Centrifugation was usually the method of choice since it offers the least chances for contamination or alteration of sample characteristics. One of the main problems associated with centrifugation occurs with oil-containing samples where the formation of an upper oil layer and fine emulsions can make separation and collection of the aqueous phase difficult. Another problem is that different centrifugation conditions will result in different amounts of residual suspended material in the aqueous sample. Toxicity associated with this suspended material could affect the apparent toxicity of the aqueous extract. Filtration always risks having toxic contaminants leach from the filter into the extract or having organic material from the sample adsorbing to the filter. Therefore, if their use cannot be avoided, the filters used should be made of an inert a material as possible, prewashed, and appropriate controls should be run.

Carrying out an aqueous extraction on a solid sample significantly increases the total analysis time as compared to performing the Microtox test alone. However, the overall cost and complexity remains low. We recommend that an attempt to standardize procedures be made in order to facilitate comparison of toxicity results across studies. Ideally this would involve the standardization of solvent systems for particular classes of toxicants or sample types (in order to minimize differences arising from changes in pH, salinity, and solvent ratio), mixing times (which would ensure the extraction approaches equilibrium), and separation/collection procedures (in order to control the amount of residual suspended particulates or emulsion in the aqueous sample to be tested).

Organic Extraction

The apparent toxicity of a solid sample can be markedly different if an organic extraction is carried out rather than an aqueous one (Dutka and Kwan 1988). This should not be surprising since many toxic organic pollutants are hydrophobic. Though bound to the solid matrix and poorly soluble in water, these compounds can enter the food chain through the action of microbial metabolism/activities (Swartz and Lee 1980). Their detection is therefore of great interest in determining and evaluating their potential impact on the environment.

The organic extraction procedures reviewed generally included the following five steps.

1) Removal of water from the sample.
2) Addition of an organic solvent.
3) Mixing and collection of the organic phase (repeating steps 2 and 3 as necessary).
4) Drying the organic phase and concentrating it to minimal volume.
5) Exchanging the extract into a solvent suitable for Microtox testing.

Removal of water

Organic extractions were performed on a fresh homogeneous sample, or on a sample that had previously been subjected to aqueous extraction (Dutka and Kwan 1988). Water was removed from

Table 2. Procedures for organic extraction for Microtox bioassay of solid samples.

Sample Type (amount)	Remove Water by	Solvent (no. of extractions x volume)	Mix with (time)	Separate organic phase	Concentrate	Solvent exchange	Reference
aqueous extracted sediment (100 g dwt)	freeze drying	MC (1 x 250 mL)	wrist-action shaker (24 h)	settle overnight, filter through Na_2SO_4	N/A	add 1 mL DMSO, rotovap off all MC	Dutka and Kwan 1988
suspended particulate phase	cascade filter, resuspend in DDW, filter (0.2 um)	DMSO (add 0.5 mL to filter and solids in vial)	sonicate (30 min)	N/A	N/A	N/A	Rao and Kwan 1990
sediment (100 g)	wash with methanol	MC:methanol (2:1), (2 x)	tumbler (18 h, 6 h)	combine methanol with MC:methanol, wash with DDW, decant, filter MC through Na_2SO_4	concentrate to 25 mL	exchange 100 uL concentrate into carrier solvent (MC, acetone, methanol, ethanol, or DMSO) final volume 3 mL	Schiewe et al. 1985
sediment (10 g dwt)	Na_2SO_4	MC (3 x 100 mL)	tumbler (16 h, 6 h, 16 h)	decant	concentrate to 10-15 mL (60°C), concentrate to 1 mL on testube heater	exchange concentrate with hexane, 3 mL, exchange 100 uL hexane to 3 mL ethanol	Tetra Tech Inc. and E.V.S. Consultants Inc. 1986
sediment (10 g dwt)	Na_2SO_4	MC (3 x 100 mL)	sonicate (15 min)	decant, filter through Na_2SO_4	concentrate to 6 mL (85°C), concentrate to 1 mL (20-30°C) with N_2	exchange 100 uL concentrate to ethanol, adjust to 3 mL final volume	True and Heyward 1990

dwt (dry weight), DDW (distilled, deionized water), DMSO (dimethyl sulfoxide), MC (methylene chloride), N/A (not applicable) the sample by pouring off as much excess liquid as possible and adding anhydrous sodium sulfate (Tetra Tech, Inc. and E.V.S. Consultants, Inc. 1986; True and Heyward 1990), freeze drying (Dutka and Kwan 1988), or washing with methanol (Schiewe et al. 1985).

Addition of anhydrous sodium sulfate is simple and effective. Freeze drying is slow, requires a significant increase in sample handling, and will result in the loss of volatile components from the sample. Washing with methanol to remove water will also result in some sample loss even if the methanol wash is later scrubbed with the organic solvent.

Addition of an organic solvent

The organic solvent most commonly used was methylene chloride (MC). A variety of solid:solvent ratios were used as summarized in Table 2. Serial extractions of the sample were usually carried out to maximize extraction efficiency. Alternatively, Shiewe et al. (1985), after having dried the sample with a methanol wash, extracted it with MC:methanol (2:1). The organic extract was then combined with the methanol wash and this solution was washed with water to remove the methanol and cause the majority of the hydrophobic components to partition into the MC.

MC is a commonly used organic solvent since it is known to have good solubilizing characteristics and is highly volatile (allowing for rapid concentration). Drawbacks to its use are that all traces of water must be removed from the extract (hence the use of anhydrous sodium sulfate), it is toxic to the Microtox test organism, and it has been shown to produce carcinogenic and teratogenic effects in mice with chronic exposure (Caledon Laboratories Ltd. 1988). Since MC is both immiscible with water and toxic to the Microtox test organism, it is poorly suited for use as a vehicle to deliver extracted toxicants to the test organism. In order to minimize the effect of the carrier solvent and better resolve the effect of the extracted toxicants on the test organism, it is necessary to exchange the extract (or a portion of the extract) from the MC into a more suitable solvent.

Mixing

Mixing was accomplished by shaking (Dutka and Kwan 1988; Tetra Tech, Inc. and E.V.S. Consultants, Inc. 1986), tumbling (Tetra Tech, Inc. and E.V.S. Consultants, Inc. 1986; Schiewe et al. 1985) or sonication (Rao and Kwan 1990, True and Heyward 1990). Extraction times ranged from 6-24 hours for shaking and tumbling, to 15 to 30 minutes for sonication.

Mixing times should be standardized in order to be reasonably sure that equilibrium conditions are attained for the toxicants under investigation. Sonication has the advantage of being extremely rapid. Unfortunately, gritty or sandy samples cause excessive damage to the expensive probe tips, drastically reducing their lifetime and possibly contaminating the sample with trace metals such as chromium which is itself toxic.

Drying and concentration

The pooled organic fractions were filtered through anhydrous sodium sulfate to remove any remaining traces of water and concentrated to low volume (Dutka and Kwan 1988; Tetra Tech, Inc. and E.V.S. Consultants, Inc. 1986; True and Heyward 1990).

The necessity of concentrating the sample makes the loss of some of the more volatile components of the sample inevitable. This loss can be minimized by using a solvent with a low boiling point and by avoiding excessive heating of the sample.

Solvent exchange

Ethanol and dimethyl sulfoxide (DMSO) have generally been the carrier solvents of choice. Shiewe et al. (1985) tried using five different carrier solvents: MC, acetone, methanol, ethanol, and DMSO (in order of decreasing toxicity). They reported that upon exchanging MC with DMSO, unexpectedly high toxic effects were observed. This increase in toxicity could not be explained by residual MC or by toxic impurities present in the solvents used and was attributed to unidentified side products formed during the solvent exchange procedure. They therefore recommend ethanol as the solvent vehicle of choice. In cases where the sample size is extremely small, it may be possible to eliminate many of the steps outlined above by extracting directly with a small volume (e.g. <1 mL) of DMSO (Rao and Kwan 1990). The extract can then be diluted with water and assayed for toxicity.

In an ideal situation, the extraction solvent would be used to deliver the toxicant to the test organism. This would require that it fulfil the criteria of being able to solubilize the toxicants, highly volatile (for rapid concentration with minimal sample loss), miscible with water, and minimally toxic to the test organism. MC is often used as an extraction solvent because it has superior characteristics with respect to the first two criteria; unfortunately it does not meet the latter two. This necessitates exchanging the extract into a carrier solvent which can solubilize the extract, is miscible with water, and is minimally toxic to the test organisms. This step adds significantly to the time and manipulation required when carrying out organic extractions.

Organic extraction of a solid sample generally takes significantly longer than the Microtox test itself. The increase in sample manipulation and equipment required is considerable as well. Variations in solvent systems, mixing, concentration, solvent exchange and proportion of the total extract subjected to Microtox testing makes it impossible to compare relative toxicity of samples across the different procedures used.

These points negate some of the advantages of the Microtox test, but it remains a comparatively inexpensive and rapid method of assessing the toxicity of hydrophobic components of solid samples. It would be advantageous if a solvent system could be developed which did not require exchanging the extract into a carrier solvent. Standardization of optimal extraction procedures for specific classes of toxicants and sample types of general interest would be extremely useful to investigators who wish to employ the Microtox method of analysis and would reduce the current difficulties in the comparison of relative toxicities across studies.

Reporting Results

Most studies followed the convention of reporting an effective concentration based on percent response at a specific time (e.g. 15 minute EC_{50}), as is done with aqueous samples. No other parameters are routinely reported.

Unfortunately, reporting a 15 minute EC_{50} for a solid sample only allows comparison of relative levels of toxicity within the context of that particular study and not across studies. This is because observed toxicity levels in different studies will be related to such parameters as the partition coefficient of the toxicants in the extraction solvent used, the solid:solvent ratio used, whether or not equilibrium was achieved during extraction, and whether any concentration, dilution or loss of toxicant occurs during sample manipulation. Standard terminology and explicit documentation in reporting findings is essential to enhancing the comparison of data across studies which use similar procedures. Dutka and Kwan (1988) moved in this direction when they reported toxicological data for aqueous extracts as being based on a 1:1 (sediment:solvent) ratio and related their EC_{50} per g wet weight of sediment. They also reported toxicological analysis of organic extracts as being performed on a suspension of the extracted sediments originally suspended in 1.0 mL of 100% DMSO and then diluted to 1% DMSO with distilled water. It is essential that such terms of reference be easily understood and, where feasible, should relate to some basic unit of measure (e.g. per g dry weight for samples extracted with aqueous solvent).

Recommendations for standardization of procedures

After reviewing the methods employed in adapting the Microtox assay to solid samples, several recommendations can be made with respect to improving the procedures for extraction of solid samples and for making maximum use of data produced in any future studies.

1) Microtox is becoming widely used as a standard microbial bioassay for toxicity in aqueous samples. Its popularity stems from the fact that it is relatively inexpensive, extremely rapid, and simple to perform. Any attempt to improve the methods for routine use of Microtox in the toxicological analysis of solid samples should consider keeping the cost, time, and complexity of the extraction procedures to a minimum.

2) It is unlikely that a single procedure could be made to serve the needs of all users and be applied in all situations (e.g. an extraction procedure using an aqueous solvent would not accurately reflect the presence of toxicants which are hydrophobic). The best alternative would be to have a few rational, standardized methods which are applicable to the analysis of compounds which are in the main stream of current interest (e.g. those toxicants which have been designated as priority pollutants). Investigators who are unable to conform their methods to documented standards could then at least make informed decisions on required modifications for their particular area of study.

3) Adopting standard methods would enable the use of standard terminology when reporting findings. The successful development of such conventions would then lend greater meaning to semi-quantitative scales used to relate the degree of toxicity observed in solid samples across studies (e.g. for 15 min EC_{50} of <25% = extremely toxic, 25-50% = very toxic, 51-75% moderately toxic, 76-100% = slightly toxic, and >100% non-toxic (no toxic response)).

4) Investigations into the improvement and standardization of aqueous extraction procedures should include optimizing solvent systems (for particular classes of toxicants or sample types) and developing appropriate standards for mixing times and separation/collection procedures.

5) Investigations into the improvement and standardization of organic extraction procedures should attempt to minimize the sample handling and manipulation (e.g. develop a solvent system that will eliminate the need to carry out a solvent exchange), optimize conditions to ensure adequate extraction of the toxicants of interest, and define optimal solvent exchange procedures, if that is required. The possibility of significant toxicity residing in the highly volatile components of a solid sample should also be investigated.

In conclusion, there is no ideal method of extracting and delivering the toxins in a solid sample to the Microtox test organisms. However, adopting standard methods and explicitly documenting methods that deviate from those standards will greatly aid an investigator's ability to assess the toxicity of solid samples.

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THE SOLID PHASE ASSAY: A NEW MICROTOX® TEST PROCEDURE. K-K. Tung, M. G. Scheibner, C. C. Walbourn, Microbics Corporation, 2232 Rutherford Road, Carlsbad, CA 92008, U.S.A. (619-438-8282)

A modification of the Microtox test has been developed which simulates environmental exposure conditions from solid phase samples. This new test protocol, called the Microtox Solid Phase Test, takes advantage of direct contact between solid phase sample constituents and the test organisms. Consequently, soluble and bound chemicals are able to interact with test organisms providing a realistic exposure route not always available with sample extracts. This new method has been evaluated using EPA prepared synthetic soil samples spiked with combinations of organics and metals. A comparative study demonstrated good correlation with this new method and a benthic bioassay on 50 tested marine sediment samples. The new solid phase test procedure will be described and comparative data presented. In addition, data from precision studies and a comparison with the standard Microtox procedure will be reviewed.

CHRONIC EFFECTS OF THIOCYANATE IN FATHEAD MINNOWS: A MODEL FOR THE EFFECTS OF A WATERBORNE ANTITHYROID ON REPRODUCTION IN FISH

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INTRODUCTION

Previous observations on the antithyroidal effects of thiocyanate (SCN$^-$) have been derived almost entirely from mammalian research, and the presence of an antithyroidal effect of SCN$^-$ in fish remains to be verified (Eales and Shostak, 1983). SCN$^-$ occurs in aquatic systems as a by-product in the detoxification of HCN in industrial processes (Ingles and Scott, 1987), or during the metabolic detoxification of waterborne HCN or cyanide from cyanogenic dietary precursors. In traditional endocrinological investigations into the effects of antithyroids in fish, nominal levels of antithyroid compounds have been administered by intraperitoneal injection (Eales and Shostak, 1983; Singh et al., 1977) or immersion in pharmacological doses (Goldsmith, 1944), without actual determination of antithyroid levels in the organism. Extrapolation from physiological effects of antithyroids to whole animal responses have also been limited.

The objectives of our study were to to determine the no observed effect concentration (NOEC) of waterborne SCN$^-$ for reproduction in the fathead minnow (*Pimephales promelas*), and to develop a laboratory model for the study of effects of antithyroids on reproduction in freshwater fishes. The experiment was designed to modify the endocrinological approach of antithyroid investigation to one more suited for chronic toxicity testing.

MATERIALS AND METHODS

Late juvenile fathead minnows (1.13 g) were exposed to either 0.06, 1.1, 7.3, 16.6, or 32.6 mg SCN$^-$/L for 21 d. Spawning substrates were then introduced into the system and reproductive response to waterborne SCN$^-$ was monitored for 103 d by measuring egg production, time to first spawn, fertilization and hatching rates, and survival and maturation of F_1 fish. Plasma SCN$^-$ levels and goitre development were monitored in relation to reproductive success. Reproductive response of fish during a recovery period after the removal of SCN$^-$ was also examined.

RESULTS

The most marked effect of SCN^- exposure on maturing juvenile fathead minnows was the delay or lack of development of secondary (2°) sexual characteristics and normal spawning behaviour. Fish exposed to 32.6 mg SCN^-/L did not develop secondary sexual characteristics, nor exhibit spawning behaviour. Fathead minnows exposed to 16.6 mg SCN^-/L exhibited incomplete development of 2° sexual characteristics and no spawning behaviour. Fish exposed to 0.06, 1.1, or 7.3 mg SCN^-/L developed normal 2° sexual characteristics and followed normal patterns of courtship and spawning.

Egg production was not significantly different between controls and fish exposed to 1.05 mg SCN^-/L, but the numbers of eggs produced declined significantly at 7.3 mg SCN^-/L. No eggs were produced by fish exposed to the two highest concentrations of SCN^-. Fertilization rates were not affected by waterborne SCN^- concentration.

Mortalities were only apparent at 32.6 and 16.6 mg SCN^-/L with 70% and 30% dying, respectively. Feeding response also appeared to be diminished at 35 mg SCN^-/L. Fathead minnows exposed to 7.3, 16.6, or 32.6 mg SCN^-/L developed overt goitre, which was confirmed by histological observation.

F_1 juveniles of adults exposed to 0.06 and 1.1 mg SCN^-/L survived to sexual maturity and produced viable offspring. The progeny of adults exposed to 7.3 mg/L survived, but produced no eggs, while control eggs incubated, hatched, and reared at 16.6 and 32.6 mg/L showed signs of goitre development at early juvenile stages and all were dead by about 8 weeks.

During the recovery phase of the study, all adults previously exposed to SCN^- produced eggs. Egg production was reduced in groups exposed previously to 16.6 mg SCN^-/L.

DISCUSSION

The primary effects of chronic SCN^- exposure on reproduction of fathead minnow appear to be on the maturation and spawning of late juvenile/adult fish, probably mediated through the antithyroidal activity of SCN^-, with minimal effects on egg fertilization. Fertilization rates were not affected by SCN^- when eggs were spawned, suggesting that SCN^- concentrations up to 7.6 mg/L do not impair the fertilization process in fathead minnows.

The most striking morphological effect of SCN^- exposure on both adult and juvenile fathead minnows was the development of overt goitrous nodules in the branchial region. Subsequent histological examination confirmed the presence of a diffuse, hyperplastic, hypertrophic goitre, which appeared to increase in severity with SCN^- dose, paralleling reduced reproductive effort.

Waterborne SCN^- exposure delayed sexual maturation of juvenile fathead minnows and reduced egg production by adult fish. Since thyroidal function has been linked with gonadal activity in teleost fishes (Sage, 1973; Cyr and Eales, 1988), chemical interference with normal thyroid function would likely affect normal sexual maturation and spawning processes. Antithyroidal agents have been shown to inhibit the development of 2° sexual characteristics and gonadal maturation in a number of fish species (Canton et al., 1983; Mukerjee, 1975; Goldsmith et al., 1944), as well as inducing thyroid hyperplasia.

Although not thoroughly understood, the reproductive effects of antithyroids in teleosts may be due to either indirect or direct gonadal effects (Singh et al., 1977). Waterborne SCN^- exposure leads to increased plasma SCN^- levels, which may result in the competitive inhibition of waterborne and thyroidal I^- uptake. By interfering with I^- uptake from the water (De Renzis, 1975; Epstein et al., 1975), from the plasma by thyroid (Wolff,1964), and subsequent I^- organification (Yamada et al., 1974), SCN^- ultimately reduces triiodothyronine (T_3) and thyroxine (T_4) production. Through the negative feedback of T_4 on the pituitary, Thyroid Stimulating Hormone (TSH) production is increased. TSH acts upon the thyroid follicular cells to incorporate more I^- for T_4 and T_3 production and the net result is often visible as goitre, the hypertrophy and/or hyperplasia of thyroid follicular cells (Leduc et al., 1982). Indirect antigonadal effects may result from the production of excess TSH at the expense of pituitary gonadotropin hormone (GtH) production.

Direct antigonadal effects may result from the reduced uptake of essential elements such as phosphorous and iodide by the gonads. Ovarian accumulation of these elements for the development of eggs and survival of young may be competitively inhibited by plasma SCN^- (Singh et al., 1977; Singh, 1969; Lindsay et al., 1966).

Historically, antithyroids have been administered to fish either by ip injection or immersion in a static system at pharmacological, not physiological doses. Nominal doses of antithyroids are calculated, but the actual amount of chemical in the body of the test organism is lacking. The quantification of plasma SCN^- levels in fathead minnows in this study offers a unique situation where internal SCN^- levels can be related to biological response, rather than using waterborne SCN^- levels as a surrogate. Plasma SCN^- levels begin to rise in fish exposed to 7.6 mg SCN^-/L, to a level of about 2.5 mg SCN^-/L, with a bioconcentration factor (BCF) <1. At 16.6 mg/L, the SCN^- influx was substantial (45 mg/L) and BCF increased to 2.7, while fish exposed to 32.6 mg/L had elevated plasma SCN^- levels (450 mg/L), with a BCF of 13.8, apparently having greatly reduced physiological control over SCN^- uptake and excretion. Egg production appears to decrease when plasma SCN^- concentrations of about 2.5 mg SCN^-/L are attained.

The effect of SCN^- appears to be transient, as illustrated by the production of eggs during the recovery period by adults previously exposed to 16.6 mg SCN^-/L for 124 d. Removal of the SCN^- exposure has been shown to result in the recovery of exposed organisms, with the effects occurring only as long as plasma SCN^- levels remained elevated. With a half-life of about 2 d (Brown et al., in preparation) complete clearance of plasma SCN^- in fatheads exposed to 16.6 mg/L (45 mg/L SCN^- in plasma) would occur in about 8 d. The delay in the onset of egg production in these fish is likely attributable to the depuration of plasma SCN^- to a level that does not impair physiological pathways involved in reproduction, and possibly to the time required to repair damage induced by elevated plasma SCN^- levels.

In summary, gonadal maturation and spawning of juvenile/adult fathead minnows was reduced with increased plasma and waterborne SCN^- concentrations, and accompanied by an increase in goitre development. The no observed effect level for impaired reproduction in the fathead minnow is between 1 and 8 mg/L SCN^- in the water and <2.5 mg/L SCN^- in the plasma. The fathead minnow/SCN^- system has potential for investigating the reproductive effects of antithyroids, but is limited by the small size of the fish at maturation, which limit the availability of plasma for concurrent determination of plasma thyroid and reproductive hormones.

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Toxicant Uptake Across Fish Gills.

by

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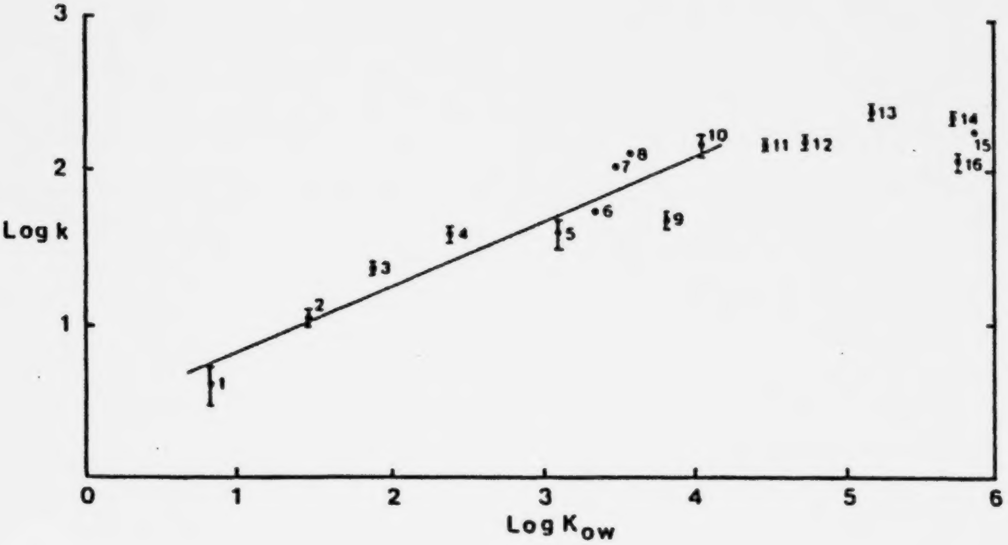
Many xenobiotic chemicals are taken up by diffusion across fish gills in a manner that parallels oxygen uptake. This study looks into the possibility that gill transfer coefficients for these compounds can be predicted from measurements of oxygen uptake.

In aquatic systems xenobiotic compounds are either surface active or enter the body of the animal. Many compounds are transferred from the water into the body either by passing directly through cells or via paracellular channels between cells. Epithelial cells are usually cemented together by tight junctions reducing paracellular transport to a minimum. Substances can pass through the cell if they are lipid soluble, if not, they must be either bound to a membrane transport molecule or pass through a limited number of small water filled channels in the membrane. In general, transport proteins in the membrane only bind specific compounds and molecules must be very small to pass through membrane channels. The surrounding lipid membrane of epithelial cells, joined together by tight junctions, forms a significant barrier around the animal to lipid insoluble compounds. It is not surprising, therefore, that the entry of xenobiotics into animals depends on lipid solubility.

If a chemical must be lipid soluble to enter an animal, then most of the chemical, once inside the animal, will be dissolved in the fat. Many xenobiotic compounds have octanol:water partition coefficients (K_{ow}) between 100 and 1,000,000. An animal may be 10% fat and if Log K_{ow} is 6, the ratio of chemical dissolved in body fat versus body water will be 10^5, assuming that K_{ow} reflects the distribution between body fat and body water. This is a reasonable assumption because Dobbs and Williams (1983) showed that fat solubility of xenobiotics varied little with the type of fat. If the chemical is in equilibrium between the water and the animal then clearly the animal will contain much more of the chemical because of body fat content. This is referred to as bioaccumulation, and in aquatic animals where the concentration of a chemical in the body is compared with that in the water, it is termed "bioconcentration". In terrestrial animals, where comparison is made, not between the animal and the environment, but between the concentration of the chemical in the food and the animal, then the term "biomagnification" is used (Connell, 1990).

There is a linear relationship between the rate of absorption by fish and the octanol/water partition coefficient of many chemicals. The higher K_{ow}, the greater the rate of influx of the compound into the fish (Fig. 1; McKim et al., 1985; Saarikoski et al., 1986; McKim and Erickson, 1990). At high values of log K_{ow}, above 4 - 6, the relationship breaks down and no further increase in rate of influx is seen with increasing K_{ow} (Fig. 1), in fact McKim and Erickson (1990) report a decreased uptake with increasing Log K_{ow} above 5.

Figure 1. The relationship between the rate of absorption (Log k) by guppies (Poecilia reticulata) from acidic water ($pH<pK_a$), and the n-octanol/water partition coefficient (Kow), for various phenolics and carboxylic acids (from Saarakoski et al. 1986).



No.	Compound	MW	log $K_{o\text{-}w}$	pK_a
1	Butyric acid	88	0.79	4.71
2	Phenol	94	1.46	10.05
3	Benzoic acid	122	1.87	4.19
4	4-Phenylbutyric acid	164	2.38	4.76
5	2,4-Dichlorophenol	163	3.08	7.85
6	2-*Sec* butyl-4,6-dinitrophenol	240	3.33	4.62
7	3,4-Dichlorobenzoic acid	191	3.46	3.7
8	2,6-Dibromo-4-nitrophenol	297	3.57	3.7
9	2,4,5-Trichlorophenol	197	3.8	7.07
10	2,4,6-Trichlorophenol	197	4.03	6.22
11	2,3,4,6-Tetrachlorophenol	232	4.45	5.46
12	Tetrachloroverathrol	277	4.7	—
13	Pentachlorophenol	266	5.15	4.71
14	Pentachloroanisol	280	5.70	—
15	2,4,6-Trichloro-5-phenylphenol	274	5.8	5.9
16	DDT	350	5.76	—
17	2,3,6-Trichloro-4-nitrophenol	242	3.93	3.08

The gills represent the major portion of the body surface area of fish and also present only a 5 to 10 µ barrier between water and blood (Hughes, 1984; Laurent, 1984) consequently most of the chemical transferred between the fish and the environment occurs across the gills. Chemicals are delivered to the gill surface by a unidirectional water flow over the gills, they diffuse across the gills and are then distributed to the tissues by the blood flow. Potentially water flow, blood flow and/or diffusion across the gills could limit uptake of the chemical by the fish. Thus transfer of chemicals across the gills can be discussed in terms of ventilation, blood perfusion and/or diffusion limitations (Fig. 2).

In general the flows of water and blood are matched to their oxygen contents, that is flow * oxygen content are the same for both blood and water. The blood contains an order of magnitude more oxygen than water and so water flow is usually an order of magnitude greater than blood flow in fish. Blood contains about 5% fat and as long as log K_{ow} is above about 2, the carrying capacity of the blood to remove the chemical will exceed the ability of the water to deliver the chemical to the gills. In addition, many xenobiotics are bound to plasma proteins (Schmieder and Henry, 1988) and any ionizable xenobiotic with a pK below 8, the pH of the blood, will be retained in the blood because gill membranes are impermeable to the ionized form. That is, binding to plasma proteins, high fat solubility and ionization will tend to keep the concentration of the undissociated form in aqueous solution in the blood low and enhance the carrying capacity of the blood to transport the compound away from the gills.

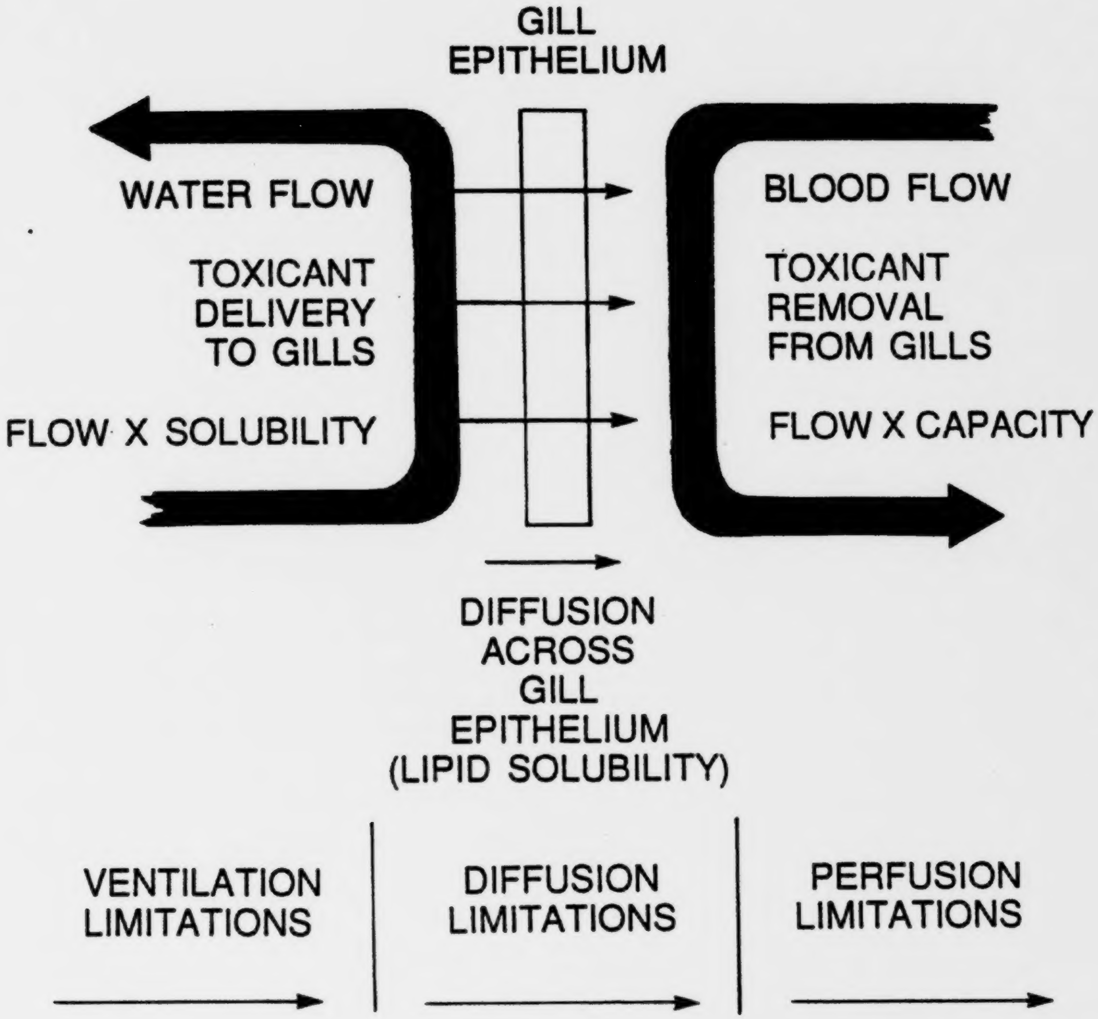

GILL
EPITHELIUM
WATER FLOW
TOXICANT
DELIVERY
TO GILLS
FLOW X SOLUBILITY
BLOOD FLOW
TOXICANT
REMOVAL
FROM GILLS
FLOW X CAPACITY
DIFFUSION
ACROSS
GILL
EPITHELIUM
(LIPID SOLUBILITY)
VENTILATION
LIMITATIONS
DIFFUSION
LIMITATIONS
PERFUSION
LIMITATIONS

Figure 2. Chemical transfer across fish gills can be limited by perfusion, diffusion and/or ventilation.

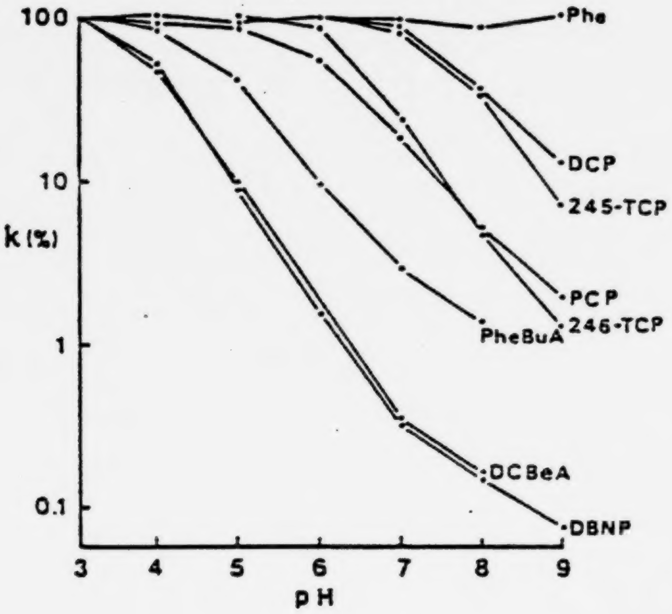
That is, transfer of xenobiotics into the fish will be dependent on water flow and/or rate of diffusion across the gills and largely independent of blood flow.

The linear increase in absorption with log K_{ow} indicates that changes in lipid and/or water solubility effects the rate of chemical uptake. The use of K_{ow} can be misleading, however, because a high K_{ow} could mean either a high lipid solubility or a very low water solubility. Dobbs and Williams (1983) showed that there was a linear relationship between water and fat solubility, but a steep inverse relationship between water solubility and log K_{ow}. That is, high log K_{ow} values were associated with very low water solubilities. The ability to deliver a chemical to the gills will depend on gill ventilation and the water solubility of the chemical, whereas diffusion across the gill epithelium will depend on the lipid solubility. At low log K_{ow} water solubility is high and the ability to deliver the chemical to the gills is high compared with the capacity of the compound to cross the gills into the blood, indicating that at low values of log K_{ow}, uptake is diffusion limited, perhaps over the range of log K_{ow} 1 to 4 (Fig. 1; Saarikoski et al., 1986). At high values of K_{ow} the reverse is true, and although fat solubility is reduced compared with compounds having a lower K_{ow} (Dobbs and Williams, 1983), water solubility is even more reduced, such that the capacity to deliver the chemical to the gill surface is much more impaired than the gill diffusing capacity and at high values of Log K_{ow} the uptake process may be limited by gill ventilation. Thus we conclude that uptake of lipid soluble chemicals tend to be diffusion limited below a log K_{ow} of about 5, but limited by gill ventilation above that log K_{ow}.

Water pH can have a marked effect on the uptake of weak acids because cell membranes are often permeable to only the undissociated forms of weak acids (Saarikoski, et al., 1986). Thus, if water pH < pK of the weak acid, absorption of the compound will be rapid but uptake will decrease with increasing pH. The actual pH at which uptake will be reduced will be related to the pK of the acid in question. Saarikoski et al., (1986) clearly demonstrated this for a number of carboxylic acids (Fig. 3). Thus the uptake of these weak acids is related to the concentration of the undissociated form rather than the total concentration of the organic compound in question. To calculate the concentration of the undissociated form in solution it is neccessary to know the pH of the solution in contact with the gills (Lin and Randall, 1990; Randall *et al*., 1990) and the pK of the compound in question. It may be difficult to determine the exact aqueous concentration of the undissociated form in water containing particulate matter that binds the compound. This is a complex problem because binding has been shown to increase with log K_{ow} of xenobiotic compounds (Black and McCarthy, 1988).

The gill diffusing capacity is a measure of the ability of the gills to transfer material, and will increase with gill area and and the inverse of gill thickness. Diffusing capacity can also change within an individual due to increased ventilation:perfusion matching, i.e. more even water flow over, and blood flow through, the gill epithelium, and a thinning of the epithelium due to elevated blood

SAARIKOSKI, J., R.LINDSTROM, M.TYYNELA, and M.VILUKSELA. 1986. Factors affecting the absorption of phenolics and carboxylic acids in the guppy (*Poecilia reticulata*). Ecotoxicology and Environmental Safety. 11: 158-173.



Phe	Phenol	10.05
DCP	2,4-dichlorophenol	7.85
245-TCP	2,4,5-trichlorophenol	7.07
246-TCP	2,4,6-trichlorophenol	6.22
PCP	Pentachlorophenol	4.71
PheBuA	4-phenylbutyric acid	4.76
DCBeA	3,4-dichlorobenzoic acid	3.7
DBNP	2,6-dibromo-4-nitrophenol	3.7

Figure 3. The relationship between rate of absorption k(%) by guppies (*Poecilia reticulata*), expressed as a percentage of that measured at low experimental pH where uptake is greatest, and the pH of the bulk water. Chemical abbreviations are defined and their respective pK_a's listed (modified from Saarikoski et al. 1986).

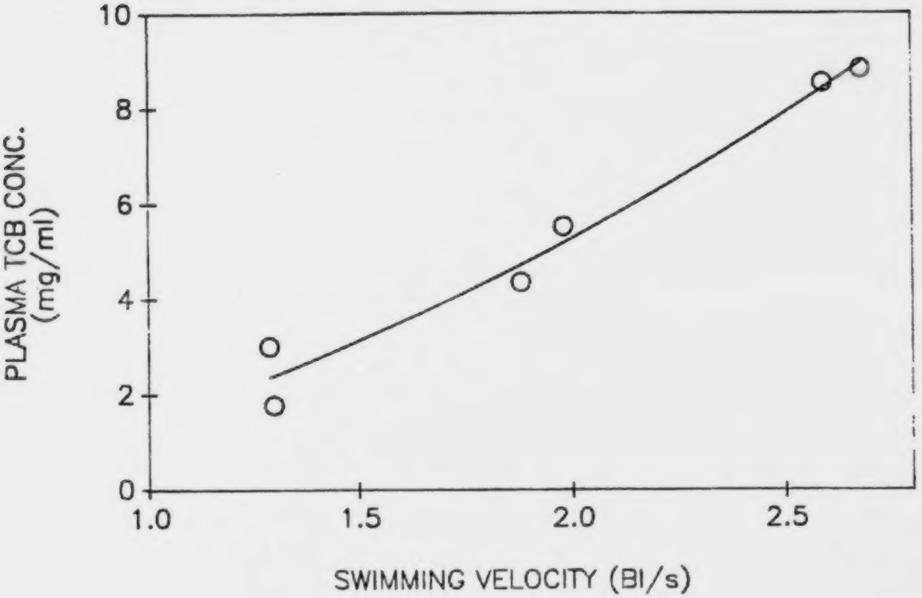
pressure. Diffusing capacity may change by a factor of 2 or 3 during exercise. Water flow over the gills also increases with oxygen uptake. Exercise therefore, can be expected to enhance the rate of uptake of a chemical because of increases in both diffusing capacity and gill ventilation. We observed this in rainbow trout, where plasma TCB levels increased with swimming speed (Fig. 3) and oxygen uptake (Fig. 4), presumably due to an increase in both water flow over the gills and gill diffusing capacity.

Measurement of gill dimensions and water flow is technically difficult and time consuming. Water flow over the gills and gill diffusing capacity is altered to maintain required levels of oxygen uptake. Oxygen uptake is, in fact, an indication of the conditions for transfer across the gills. As oxygen uptake increases so does toxicant transfer (Fig. 5; Murphy and Murphy, 1971; Rodgers and Beamish, 1981). Thus, it is posible that oxygen uptake could be used as an indicator of conditions for toxicant delivery and transfer across the gills.

Toxicant uptake increased with oxygen uptake in both goldfish and trout (Fig. 5). Comparisons of regressions of the two groups of data indicated no significant difference between the two fish species. In subsequent manipulations the data has been treated as a single set.

During the initial stages of transfer, although plasma content will be higher than that in the water, most of the chemical will be dissolved in the fat (blood of vertebrates is about 5% lipid), bound to plasma proteins (Schmieder and Henry, 1988), and/or ionized and the concentration of the undissociated chemical in aqueous solution

THE EFFECT OF SWIMMING VELOCITY ON THE UPTAKE OF 1,2,4,5 TETRACHLOROBENZENE.



THE EFFECT OF OXYGEN CONSUMPTION ON THE UPTAKE OF 1,2,4,5 TETRACHLOROBENZENE.

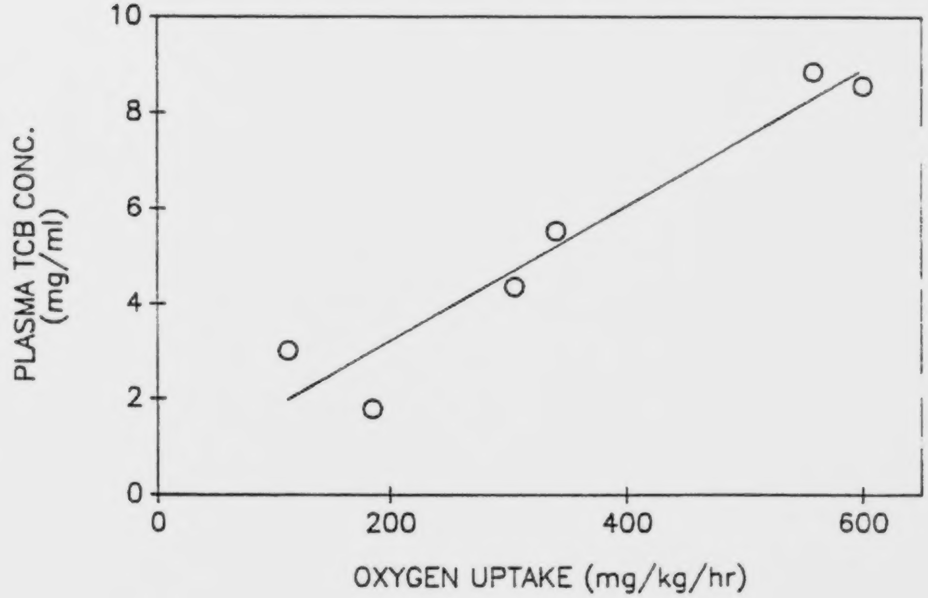


Figure 4. The relationship between plasma TCB (1,2,4,5 Tetrachlorobenzene) concentration and A) swimming speed and B) oxygen uptake in rainbow trout. (Unpublished data from Thurston,

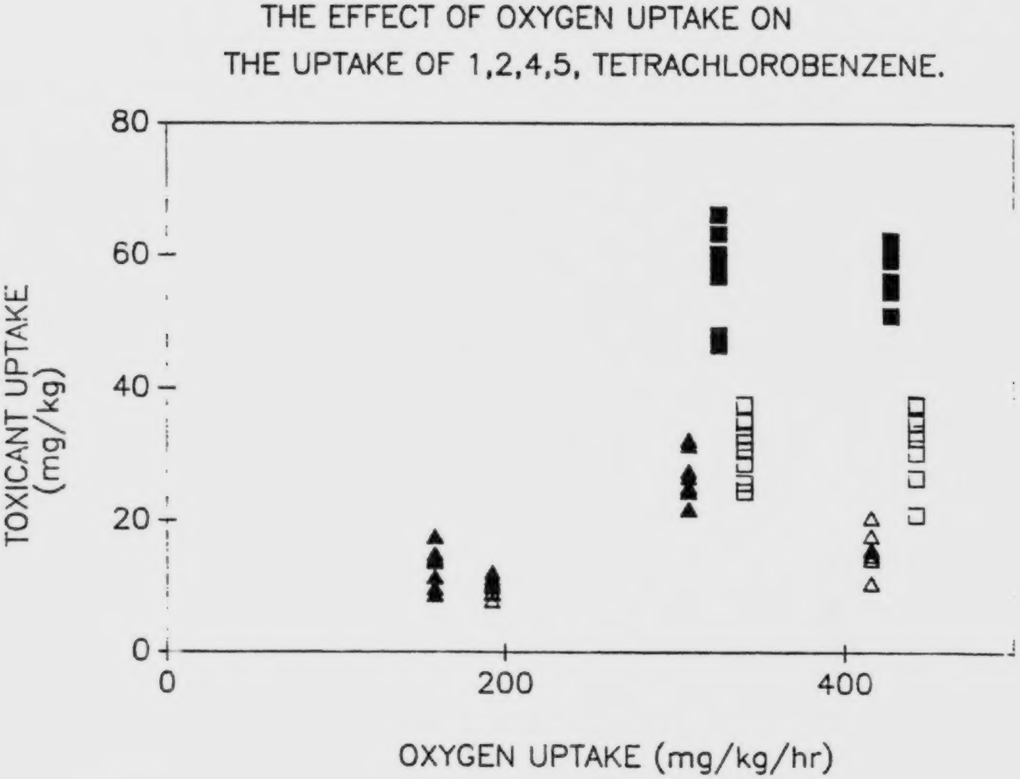


Figure 5. Total body burden of TCB in ~6g goldfish (▲) and ~9g trout (■) after two hours exposure to low (open symbols) and high (closed symbols) concentrations of TCB at different rates of oxygen uptake. (Unpublished data from Thurston, Brauner, Newman and Randall).

will be negligable, and can be assumed to be zero. During the early phases of toxicant uptake (for many chemicals the first few hours), therefore, the level in the water is a direct indication of the gradient between water and blood. We calculated toxicant uptake per unit gradient, from data presented in figure 5 and values of water toxicant concentration (Fig. 6). The slope of the regression on the data presented in Fig. 6 represents the toxicant transfer coefficient, that is the uptake of the toxicant, per unit toxicant gradient, per unit oxygen uptake, for a toxicant with a Log K_{ow} of around 5.

Toxicant transfer coefficient, λ
(mg/hr/kg per mg/L gradient, water to blood)
= [0.17 * Oxygen uptake (mg/kg/hr)] - 11.7.

Uptake rates do not change with log K_{ow} values above about 4 (Figure 1), so this relationship could be applicable to toxicant uptake for chemicals with a log K_{ow} greater than 4.3. Variations in uptake of chemicals of different log K_{ow} below 4.3 are described in Figure 1. Thus, the relationship between uptake of a chemical, with a log K_{ow} below 4.3, and oxygen uptake can be described by the following equation based on data in Figures 1 and 6.

Toxicant (log K_{ow} below 4.3) transfer coefficient =
λ * [1 - (4.3 - log K_{ow})* 0.19]

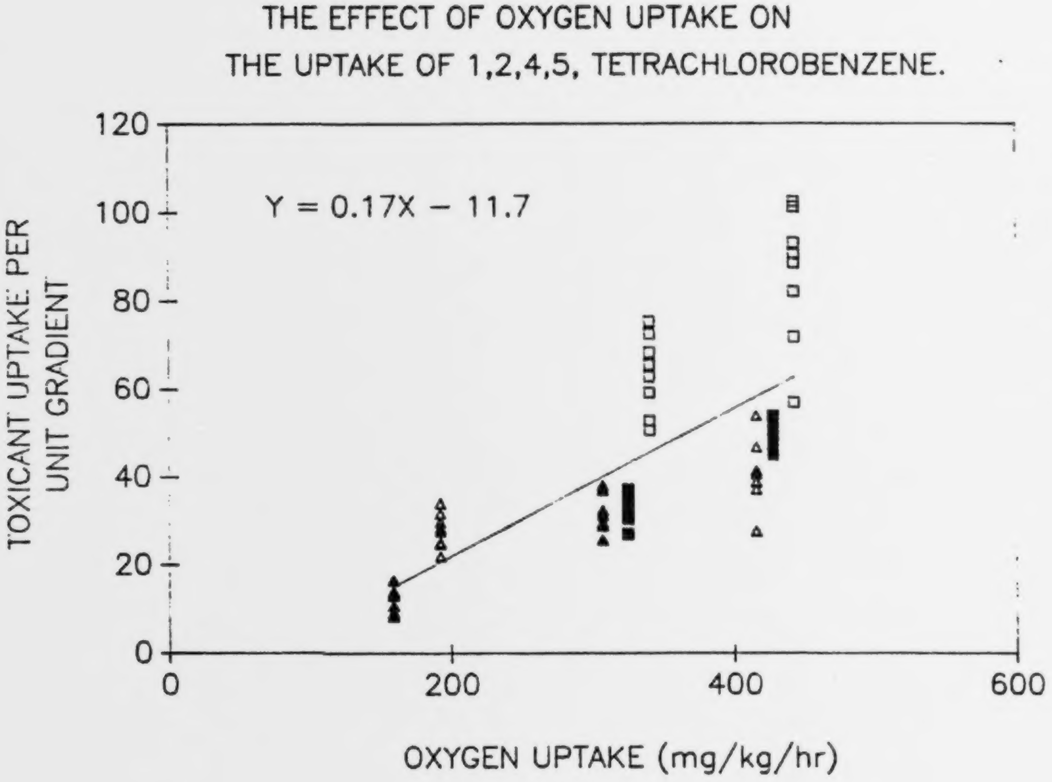


Figure 6. The relationship between the uptake of TCB and oxygen uptake of goldfish and trout, see Fig. 5 and text for further details. (Unpublished data from Thurston, Brauner, Newman and Randall).

Thus chemical uptake rate for this group of chemicals can be predicted from log K_{ow} and pK of the compound in question, the water pH and the oxygen uptake of the fish. There is a large data bank of oxygen uptake measurements in fish, and in many instances uptake data can be retrieved from the literature. It is possible, therefore, that toxicant uptake rates can be predicted for a large number of aquatic animals and a large number of chemicals, based on this simple relationship. The validity of this approach is presently being tested.

Hypoxic conditions lead to an increase in gill ventilation and gill diffusing capacity with only small changes in oxygen uptake in most fish (Randall and Daxboeck, 1984). Under hypoxic conditions one would expect a much larger increase in toxicant uptake than oxygen uptake compared with normoxic values, as observed by McKim and Goeden (1982). Fish species respond to hypoxia in different ways, so it is difficult to generalise about the effect of hypoxia on toxicant uptake, except that it will tend to be higher for a given oxygen uptake than in normoxia.

Toxicant will be taken up by the skin directly from the water and will not be transported by the blood. This uptake pathway is probably rapid and could account for a large proportion of the initial uptake, reaching equilibrium with the water far more rapidly than the rest of the body. Nothing is known of the dynamics of skin uptake but it can constitute a large proportion of the initial body burden, perhaps even as high as 40 to 50% of the initial uptake (Saarikoski et al., 1986).

This manuscript attempts to derive a transfer coefficient for uptake of xenobiotic chemicals across fish gills correlated to oxygen uptake. The rate of uptake of the toxicant by the fish will be influenced by the concentration of the undissociated form in the water and the size of the toxicant reservoir in the fish, which will depend on the size and fat content of the animal, as will the distribution of the toxicant to various tissues. The rate at which the toxicant level in the fish approaches equilibrium with that in the water will also depend on the rate of loss of the toxicant (depuration) from the fish and its rate of metabolism. None of these factors are analysed here.

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The Effect of TCMTB on Juvenile Coho Salmon (*Oncorhynchus kisutch*): Sublethal Toxicity Testing

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ABSTRACT

This study examined the sublethal toxicity of 2-(thiocyanomethylthio)-benzothiazole (TCMTB), a wood preservative used in British Columbia's lumber industry, to coho salmon, *Oncorhynchus kisutch*. Swimming performance, oxygen consumption and blood lactate levels were used as indicators of sublethal TCMTB toxicity. Assessment of swimming performance consisted of subjecting pre-exposed (48 h) fish to a series of increasing velocity increments within an annular swim chamber. An automated respirometer apparatus was used to measure oxygen consumption rates. Blood lactate levels were assayed upon termination of the respirometer trials. Reductions in critical swimming speed and elevations in blood lactate occurred in a concentration-dependant manner with thresholds of 10 $\mu g \cdot L^{-1}$ and 7.5 $\mu g \cdot L^{-1}$, respectively. A similar concentration-dependant response was not observed with respect to oxygen consumption rates.

INTRODUCTION

The antisapstain agent 2-(thiocyanomethylthio)-benzothiazole (TCMTB), is currently used as a wood treatment agent in the British Columbia lumber industry. As with other wood preservative chemicals, concern exists over the effects of TCMTB discharges into fish-bearing waters. Recent studies (G. Kruzynski, pers. commun.) indicate that TCMTB has a high acute toxicity to fish (96-h LC_{50}'s of 17.3 and 11.2 $\mu g \cdot L^{-1}$ for coho, *Oncorhynchus kisutch*, and chinook, *Oncorhynchus tshawytscha*, respectively). However, little information exists about its sublethal effects. It is imperative to examine the extent of sublethal physiological changes before a full understanding of TCMTB's ecological impact can be reached.

Sprague (1971) suggested swimming performance as an important criterion in the determination of the sublethal effects of toxicants on fish. It has been applied to a number of pollutants, including bleached kraft pulpmill effluent (Howard 1975), cyanide (Kovacs and Leduc 1982) and copper (Waiwood and Beamish 1978). This response appears to be sensitive to a number of toxic actions, one of which is impaired gas exchange across the gill epithelium (Waiwood and Beamish 1978). Oxygen consumption is a second criterion that has been suggested as an index of sublethal toxicity for fish (Sprague 1971) and one which may have a direct limiting effect on a fish's aerobic performance. Alterations in oxygen consumption rates have been used by Johansen and Geen (1990) and Janz et al. (1991) as sublethal indices of herbicide toxicity in juvenile coho salmon. Elevations in plasma lactate frequently occur in response to toxicant-induced stress and as such, have also been utilized as an indicator of sublethal stress (Heath 1987; Janz et al. 1991).

The purpose of this study was to assess the sublethal toxicity of TCMTB to coho salmon, with respect to the parameters of swimming performance, oxygen consumption and blood lactate levels. The usefulness of these measurements was evaluated to assess their applicability as sublethal toxicity indicators.

MATERIALS AND METHODS

Experimental Animals and Test Chemical

Coho salmon (presmolt) were obtained from the Capilano Fish Hatchery, North Vancouver, B.C. (mean weight 24.7 g, SEM=0.6; fork length 13.8 cm, SEM=0.1) and held under natural photoperiod in 2000-L fiberglass tanks. Dechlorinated, municipal water of pH 6.1-6.7, O_2 saturation >95% and hardness 5.2-6.0 $mg{\cdot}L^{-1}$ $CaCO_3$ was used throughout the study. Fish were maintained on Oregon moist pellets except for 24 h preceding experimental trials, when feeding was withheld.

2-(Thiocyanomethylthio)-benzothiazole (TCMTB) is the active ingredient of the commercial fungicide Woodstat 30WB, comprising 30% of the product. The remainder of the product consists of a carrier of proprietary chemical composition. Both Woodstat 30WB and the carrier are products of Buckmann Labs, Memphis, Tennessee. Sublethal TCMTB concentrations evaluated were based on a 96-h flow-through LC_{50} value of 17.3 $\mu g \cdot L^{-1}$ for juvenile coho salmon (G. Kruzynski, pers. commun.). Stock solutions of TCMTB were prepared for each experimental trial using glass-distilled, deionized water and were kept in opaque, glass flasks.

Oxygen consumption

Oxygen consumption trials were performed from 27 March - 29 June 1990 in an eight vessel, computer-controlled, intermittent-flow respirometer similar to that of Duval et al. (1981) and as modified by Johansen and Geen (1990). This respirometer design allows freshwater flushing and oxygen consumption measurements within each vessel every 30 minutes. Appropriate TCMTB concentrations were maintained in the vessels by computer control of delivery pump timing. External disturbances were minimized by blacking-out the vessels with opaque polyethylene covers. Water temperatures throughout the trials were 10.5-13.0°C and a 14 h light:10 h dark (30 min dawn and dusk, photoperiod was maintained).

Fish were weighed, placed in the 8-L vessels (5 per vessel) and allowed to acclimate under continuous-flow conditions for a 24-h period. Trials consisted of a 48 h toxicant pre-exposure period followed by a 48 h exposure period, starting and ending at 1300 hours. Fish were exposed to Woodstat 30WB giving nominal TCMTB concentrations of 5.0, 7.5, 10.0, 15.0 and 20.0 $\mu g \cdot L^{-1}$, respectively. A 0 $\mu g \cdot L^{-1}$ control and a 20.0 $\mu g \cdot L^{-1}$ equivalent carrier solution were also employed during the exposure period. Three vessels without fish also received 0 and 20.0 $\mu g \cdot L^{-1}$ TCMTB and a 20.0 $\mu g \cdot L^{-1}$ equivalent carrier, respectively, to correct for chemical oxygen demand, if any. Five replicate trials were conducted for each TCMTB treatment as well as a single carrier trial.

Oxygen consumption rates were based on the pooled values for 5 fish within a single respirometer vessel, as measured every 30 minutes. This mass-specific rate is based on the total wet weight of the five fish in each of the test vessels. These values were averaged over 24-h periods throughout the trial and means of replicate trials were taken to obtain the final values. The effect of TCMTB on oxygen consumption was tested in two ways: (1) comparison of each TCMTB exposure value with its corresponding control exposure value, and (2) comparison of the differences between pre-exposure (24-48 h) and exposure (0-24 and 24-48 h) values for each TCMTB concentration. Since fish exhibit diurnal rhythms of oxygen consumption (Brett and Zala 1975) it was preferable to compare mean values for the full 24 h cycle rather than specific values within the cycle. A characteristically elevated oxygen consumption during the initial 0-24 h pre-exposure period precluded the use of this period for statistical analysis (Figure 1).

Blood Lactate Analysis

Immediately following the final exposure cycle, 24 ml of 50% 2-phenoxyethanol in distilled water was injected into each vessel to anesthetize the fish and minimize handling stress (Janz et al. 1991). Anesthetized fish were then sampled sequentially, with a total sample collection time of 3 min per vessel. Blood was collected from the severed caudal peduncle into individual 75 µL ammonium-heparinized microhematocrit tubes. After centrifugation (5 min at 2,000 RPM), blood plasma was pooled for each treatment, deproteinated with 8% $HClO_3$ and refrigerated at 4°C until analysis. Lactate was measured enzymatically (L-lactate dehydrogenase/NADH method; Loomis 1961) using Sigma reagents.

Swimming Performance

Fish used in the critical swimming speed trials (July 1990) were pre-exposed, within the respirometer vessels, to TCMTB concentrations identical to those described above. Each 48 h toxicant exposure was preceded by a 16-24 h continuous-flow acclimation to the respirometer. Following the exposure period, fish were transferred to the swimming chamber and allowed a 4 h acclimation prior to the actual test of swimming ability. Two replicate trials, with 5 fish per trial were conducted for each exposure concentration, including the carrier solution.

The swim testing apparatus consisted of a 2470-L ovoid, fiberglass raceway tank (outside length 343 cm, depth 41 cm, raceway width 46 cm) outfitted with two variable output propulsion motors (Figure 2). Two enclosed, cylindrical testing chambers were located within each straight section of the tank. Straightener vanes, screens and contraction cones, located upstream of each test chamber, corrected rotational disturbances introduced by the propellers and smoothed the velocity profile within the test sections. Water velocity, controlled by regulating voltage output to the propulsion motors, was pre-calibrated with a portable current meter prior to the initiation of swim trials.

Critical swimming speed was measured in bodylengths per second ($bl \cdot s^{-1}$) following the procedures developed by Brett (1964). Initial velocity was $0.20\ m \cdot s^{-1}$ ($1.4\ bl \cdot s^{-1}$) and the speed was increased in increments of $0.05\ m \cdot s^{-1}$ ($0.4\ bl \cdot s^{-1}$) at 15-min intervals to the fatigue velocity. Fatigued fish were removed from the test chambers individually via a movable, screen gate, at which time fork length and weight were recorded.

Quality assurance was based on water samples, taken at each concentration on the final day of exposure, during both the oxygen consumption and swimming performance trials. Chemical analysis for active ingredient was performed by Environment Canada, Chemistry Laboratory, West Vancouver, B.C.

Differences between the mean critical swimming speed, oxygen consumption and plasma lactate of the control, carrier and treatment groups were compared using an unpaired Student's *t*-test ($P < 0.05$). A paired Student's *t*-test ($P < 0.05$) was used for statistical comparison between pre-exposure and post-exposure oxygen consumption rates within the same group of fish.

RESULTS

A mean daily oxygen consumption rate of $97\ mg\ O_2 \cdot kg^{-1} \cdot h^{-1}$ (SEM=1.2) was recorded for control fish over the entire experimental period. Oxygen uptake decreased over time during the initial pre-exposure period for all exposure concentrations. Characteristic circadian rhythms were apparent in all groups (Figure 1).

The mean oxygen consumption rate of 91 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ (SEM=1.7) for the carrier solution was not significantly different from the control, though a slight elevation to 102 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ (SEM=2.9) did occur during the 0-24 h post-exposure interval. Neither TCMTB nor the carrier compound exhibited significant chemical oxygen demand at 20 $\mu g \cdot L^{-1}$ in the vessels without fish. One mortality occurred within the 20 $\mu g \cdot L^{-1}$ TCMTB group at 46 hours into the exposure. There was no significant change in the control oxygen consumption rate during the final three 24 h periods of the experiment.

In all treatment groups the first exposure period (0-24 h) had a lower oxygen consumption rate than its paired pre-exposure value, though this effect was statistically significant only at 5 and 20 $\mu g \cdot L^{-1}$ (Table 1). During the second exposure period (24-48 h), oxygen consumption either remained at this low level (7.5 and 20 $\mu g \cdot L^{-1}$), or showed a further decrease (15 $\mu g \cdot L^{-1}$), or increased to or above the pre-exposure level (5 and 10 $\mu g \cdot L^{-1}$). Thus, at lower TCMTB concentrations (5 - 10 $\mu g \cdot L^{-1}$) recovery was possible after an initially reduced oxygen consumption rate. At TCMTB concentrations $\geq$ 15 $\mu g \cdot L^{-1}$, oxygen consumption was depressed throughout the exposure period. A simple concentration-dependant change in oxygen consumption was not apparent.

Four out of five pre-exposure values for treatment groups were significantly lower than the control pre-exposure values (Table 1). This intergroup variability is reflected also in the finding that the majority of the post-exposure values for treatment groups were lower than the corresponding post-exposure control values.

Significant elevations in plasma lactate were observed in all treatment groups exposed at and above 7.5 µg·L^{-1} TCMTB (Figure 3). Lactate levels reached a maximum (5.29 mM·L^{-1}, SEM=0.55) within the 20 µg·L^{-1} treatment group. Plasma lactate levels of the carrier fish were determined at 1.37 mM·L^{-1}, slightly lower than those of the controls (1.62 mM·L^{-1}, SEM=0.07).

The mean critical swimming speed of control fish was 6.29 bl·s^{-1} (SEM=0.07), reflecting a sustained velocity of 0.86 m·s^{-1}. After the 48 h exposure, critical swimming speeds decreased significantly with increasing TCMTB concentrations (Figure 4), starting at 10 µg·L^{-1} (*ca* 60% of the 96-h LC$_{50}$ value). Swim speed was reduced by 11, 19 and 25% for 10, 15 and 20 µg·L^{-1} TCMTB, respectively. No effect on critical swimming speed was apparent after exposure to the carrier solution (6.26 bl·s^{-1}, SEM=0.07). In the 20 µg·L^{-1} group, three mortalities occurred during the first hour of recovery from swimming to fatigue.

Results of quality assurance analyses indicated that all samples, with one exception, fell within acceptable concentration limits (*ie.*, SD $<$ 7.4 µg·L^{-1} over 5 replicate concentrations). TCMTB concentrations were below the detection limits of 5.0 µg·L^{-1} in all control samples submitted.

DISCUSSION

The results indicate that critical swimming speed, blood lactate levels and oxygen consumption rates of coho salmon are significantly affected by sublethal concentrations of TCMTB. Reductions in critical swimming speed were apparent at sublethal concentrations of 10 µg·L^{-1} (*ca* 60% of the 96-h LC$_{50}$ value) while plasma lactate elevations exhibited a lower threshold of 7.5 $\mu g \cdot L^{-1}$ (*ca* 40% of the 96-h LC_{50} value). Oxygen consumption rates, although altered, did not respond in this concentration-dependant manner. A consistent and significant reduction in oxygen consumption rates over the entire post-exposure period occurred only in the 20 $\mu g \cdot L^{-1}$ treatment group (*ca* 120% of the 96-h LC_{50} value). However, significant alterations in oxygen consumption were apparent at even the lowest concentration tested (*ca* 30% of the 96-h LC_{50} value).

The control values reported in this study suggest that the test fish were sufficiently acclimated to the experimental apparatus. The mean critical swimming speeds of 6.29 $bl \cdot s^{-1}$ for control fish are similar to those reported by Howard (1975) for fingerling coho under comparable conditions. Likewise, mean daily oxygen consumption rates of 97 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ fall between resting values of 95 and 101 mg $O_2 \cdot kg^{-1} \cdot h^{-1}$ as reported by Brett and Zala (1975) and Barton and Schreck (1987), respectively, for juvenile salmonids at similar temperatures. The early morning oxygen consumption increases and nightly decreases reflect normal daily activity patterns (Brett and Zala 1975). Plasma lactate control values are comparable to those reported by other authors (Janz et al. 1991; Andersson et al. 1988) for fish of similar size, but higher than found in larger, cannulated fish (Wood et al. 1988). Where the size of the fish makes cannulation impractical, blood must be sampled from the caudal vasculature via the severed caudal peduncle. Like blood lactate, muscle lactate increases in response to stress but attains much higher concentrations than in the plasma (Milligan and Wood 1986). Thus, although contact between blood and tissue was minimized, a small addition of muscle lactate to the blood was probably unavoidable during the sampling process. This will result in a systematic error which overestimates total plasma lactate, but will not affect the overall conclusion that elevated plasma lactate levels imply stressed fish.

Swimming performance is suggested to be directly limited by oxygen uptake and delivery (Sprague 1971). The observed reduction in critical swimming speed reflects a possible limitation in one or both of these functions. Heath (1987) postulated that critical swimming speed is particularly sensitive to impairment of oxygen transfer across the gill. Reduced aerobic performance has been demonstrated in cases where toxicants bind or absorb to gill epithelium (Howard 1975), clog lamellae (MacLeod and Smith 1966) and cause structural gill damage at the microscopic level (Waiwood and Beamish 1978).

Elevations in the concentration of blood lactate can be indicative of anaerobic metabolism often associated with acute physiological stress (Heath 1987). Anaerobic metabolism and subsequent increases in lactate would be expected if an impairment of oxygen flux from the water to the blood was occurring under TCMTB exposure. However, it can not be clearly determined whether or not the observed increases in blood lactate were due to stress-related adrenergic stimulation of glycolysis or due to the direct effect of the toxicant-induced hypoxia on muscle tissues. Under the former situation the presence of TCMTB elicits a stress response triggering a release of catecholamines into the blood. Glycolysis, and thus blood lactate, increases in response to the elevated catecholamine levels (Mazeaud et al. 1977). In the latter case, impairment of oxygen transfer to the tissues is reflected in a proportion of the metabolic demand for ATP being supplied through anaerobic as well as aerobic processes.

Although the reductions in critical swimming speed and elevations in plasma lactate are indicative of impaired respiratory function, this is not reflected in the measured oxygen consumption rates. In support of this finding, Heath (1987) suggested that the amount of respiratory impairment which would be unlikely to affect a fish's ability to function in the environment at rest will undoubtedly have an inhibitory effect on active performance. The absence of a measurable, concentration-dependant reduction in resting oxygen consumption rates indicates that this parameter was not as sensitive to disturbed respiratory integrity as was critical swimming speed.

The three parameters used in this study vary with respect to their usefulness as indicators of sublethal toxicity. Besides being difficult to interpret, measurement of oxygen consumption is relatively insensitive when effect thresholds are compared to either swimming performance or blood lactate. The variability in oxygen consumption rates between untreated groups can, in some cases, be greater than the overall response to the toxicant itself. Extending the acclimation period further in an attempt to lessen this variability could lead to starvation-related artifacts in the oxygen consumption data. Although analysis of blood lactate concentrations is a rapid, straight-forward and highly sensitive approach to measuring toxicant-induced stress, it does appear to have limitations. As an indicator of acute physiological stress, blood lactate is responsive to a variety of extraneous disturbances, including handling (Wedemeyer 1972). Caution must be taken to separate the handling stress from that of the toxicant. Tissue injury during sample collection may also lead to non-toxicant related elevations in blood lactate levels. Finally, the biological significance of increased blood lactate levels may not be great. Impairment of swimming performance, in contrast, is an ecologically relevant response to a toxicant, particularly for upstream migrating fish. A modified version of the swimming apparatus used in this study could prove useful for *in situ* examination of the effects of sublethal toxicant exposures on swimming performance.

In summary, critical swimming performance was found to be a sensitive indicator of sublethal TCMTB toxicity, with impairment occurring at concentrations as low as 10 $\mu g \cdot L^{-1}$. Alterations in blood lactate and oxygen consumption rates, while providing useful information on physiological changes associated with sublethal TCMTB exposures, are less meaningful indicators of sublethal toxicity.

ACKNOWLEDGEMENTS

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Table 1. Oxygen consumption (MO_2) rates (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) for juvenile coho salmon prior to and during exposure to TCMTB.

	TCMTB Concentration ($\mu g \cdot L^{-1}$)											
	Control X(SEM)	N^{d}	5.0 X(SEM)	N	7.5 X(SEM)	N	10.0 X(SEM)	N	15.0 X(SEM)	N	20.0 X(SEM)	N
Pre-Exposure (24-48 h)	92.34 (2.19)	5	80.76^{b} (1.92)	5	83.52^{b} (2.23)	5	76.08^{b} (1.74)	5	82.14^{b} (2.67)	4	97.56 (2.62)	5
Post-Exposure (0-24 h)	89.70 (1.97)	5	75.06^{ab} (2.11)	5	79.38^{b} (2.31)	5	73.68^{b} (1.93)	5	77.22^{b} (2.56)	4	83.94^{ab} (1.97)	5
Post-Exposure (24-48 h)	95.46 (2.39)	5	92.38^{ac} (3.67)	5	76.62^{ab} (2.60)	5	78.60^{b} (2.40)	5	70.86^{abc} (1.96)	4	84.30^{ab} (2.34)	5

[a] Significant difference in comparison with own pre-exposure MO_2 value ($P < 0.05$, paired Student's t-test).

[b] Significant difference in comparison with corresponding control MO_2 value ($P < 0.05$, unpaired Student's t-test).

[c] Significant difference in comparison to own MO_2 value for preceding (0-24 h) post-exposure period ($P < 0.05$, paired Student's t-test).

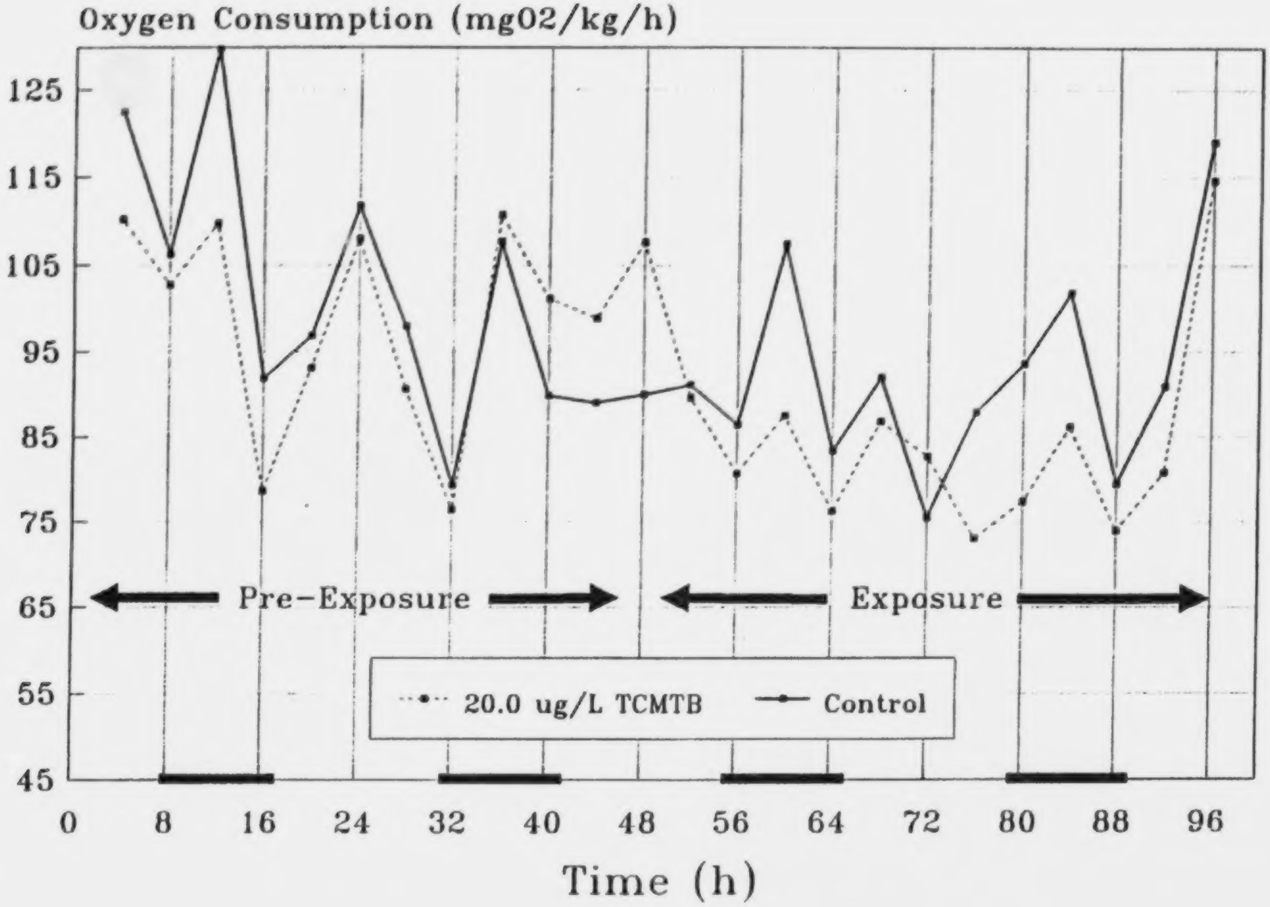
[d] N is number of experimental replicates, with each replicate representing 48 single MO_2 measurements for 5 pooled fish.

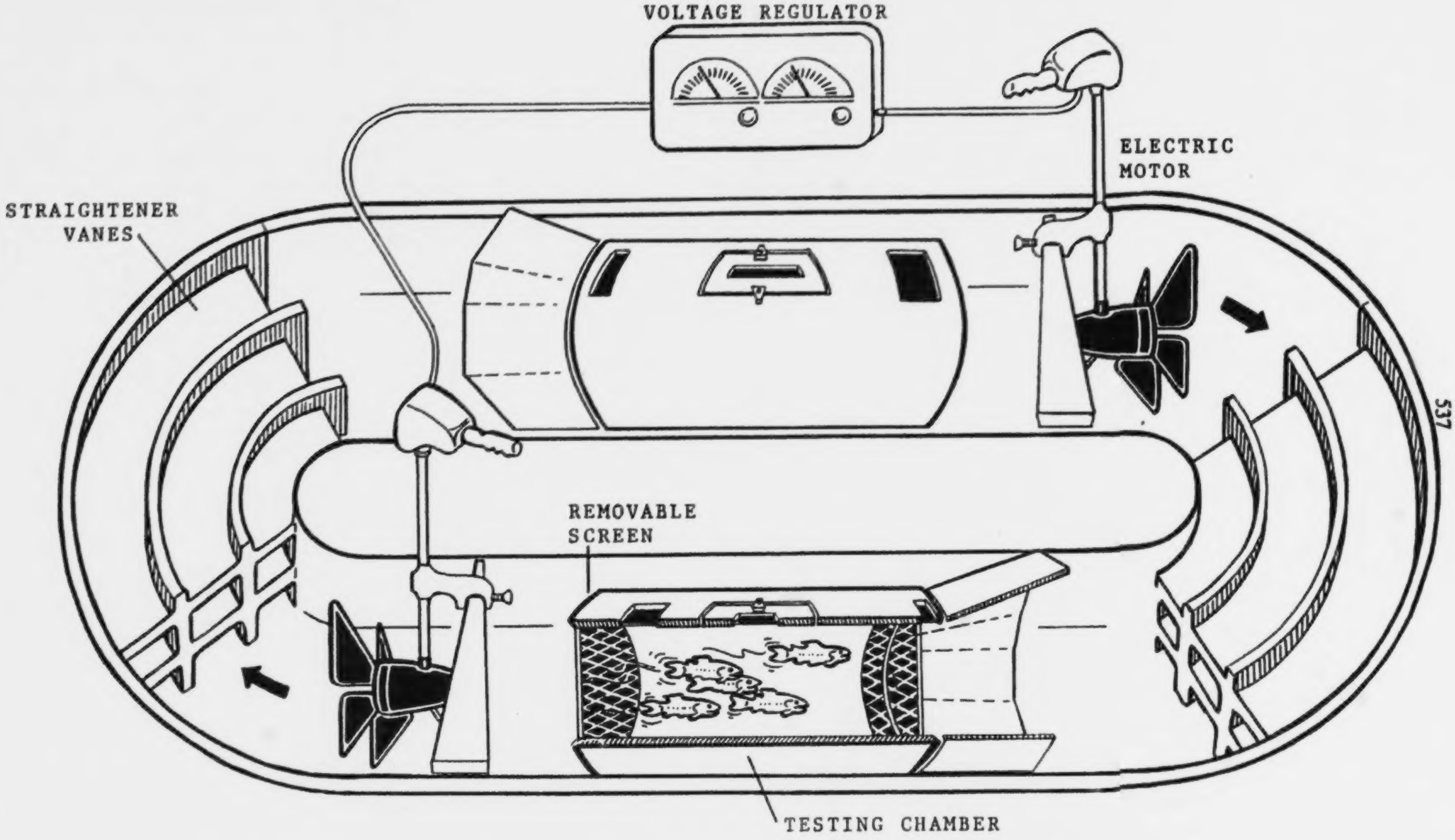
Figure 1. Daily oxygen consumption patterns of juvenile coho salmon exposed to nominal concentrations of TCMTB. Dark bands on the time axis represent the "lights off" portion of the photoperiod.

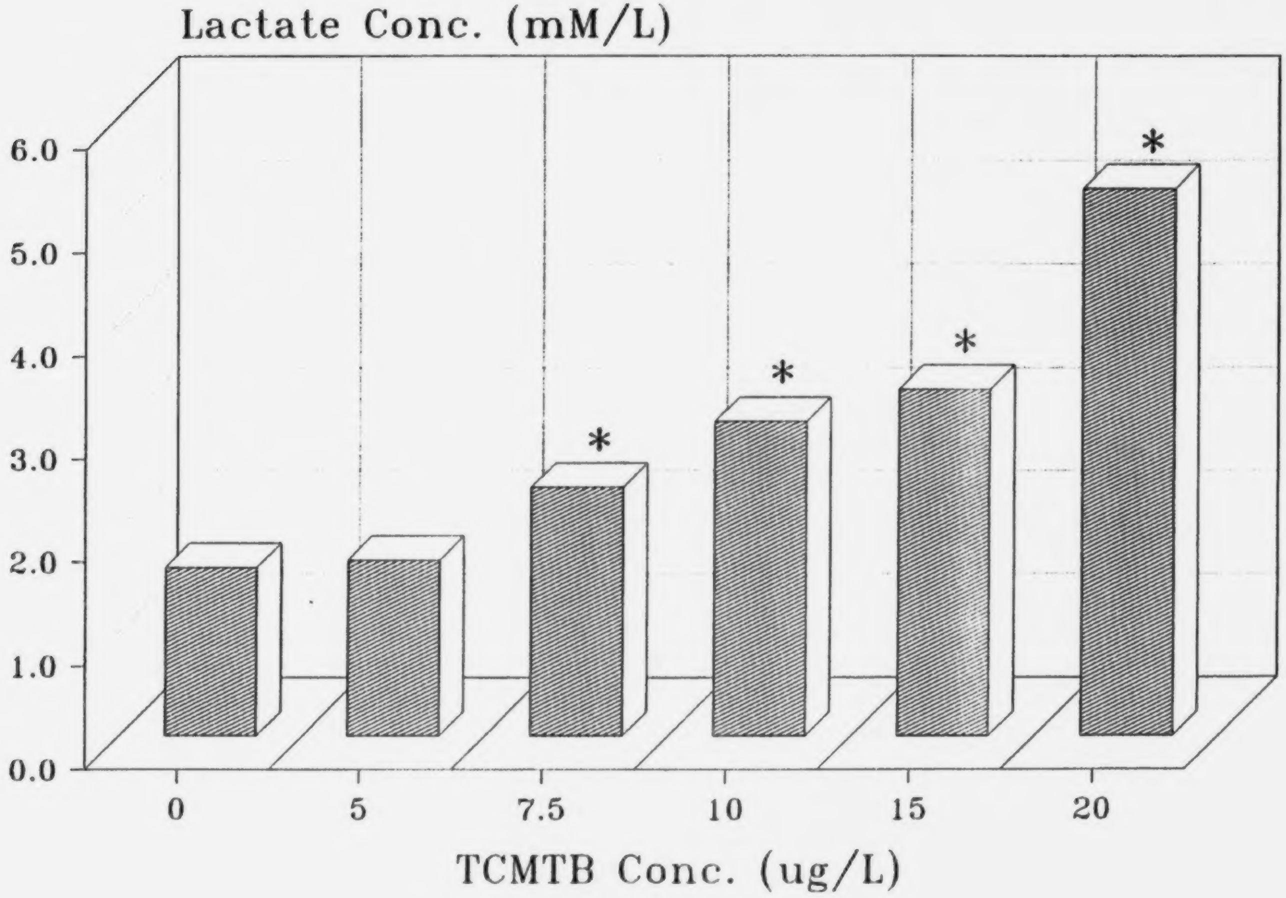
Figure 2. Diagrammatic representation of the apparatus used for examining critical swimming speed in fish.

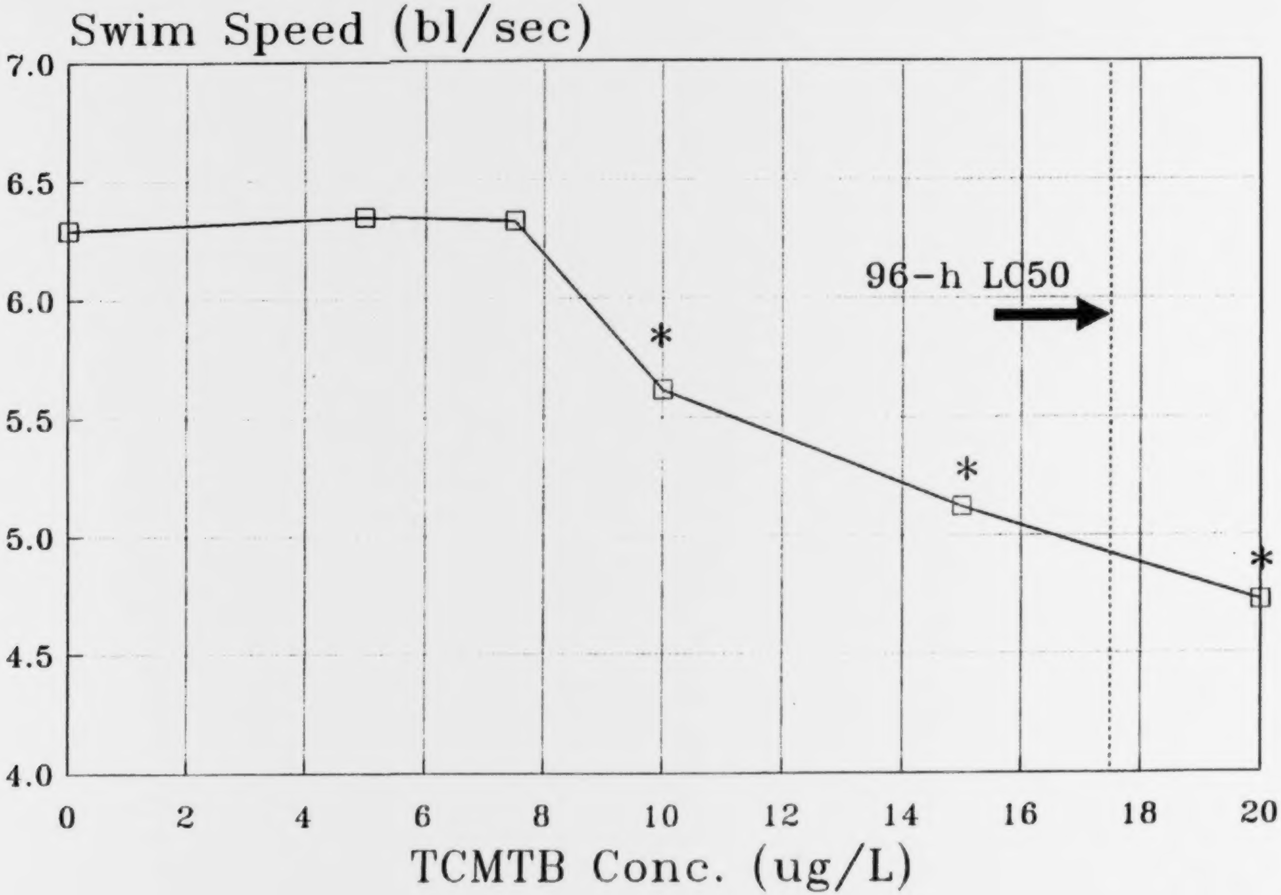
Figure 3. Blood lactate concentrations of juvenile coho salmon exposed to TCMTB for a 48 h period. Asterisks indicate significant differences ($P < 0.05$, Student's *t*-test) between control and treatment groups. N = 5 replicates, with each replicate representing a pooled value for 5 fish.

Figure 4. Critical swimming speeds of juvenile coho salmon following a 48 h TCMTB exposure. Asterisks indicate significant differences ($P < 0.05$, Student's *t*-test) between control and treatment groups. N = 10 fish per treatment group.


Oxygen Consumption (mgO2/kg/h)
125
115
105
95
85
75
65
55
45
0
8
16
24
32
40
48
56
64
72
80
88
96
Time (h)
Pre-Exposure
Exposure
20.0 ug/L TCMTB
Control


VOLTAGE REGULATOR
ELECTRIC MOTOR
STRAIGHTENER VANES
REMOVABLE SCREEN
TESTING CHAMBER


Lactate Conc. (mM/L)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
*
*
*
0
5
7.5
10
15
20
TCMTB Conc. (ug/L)


Swim Speed (bl/sec)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
2
4
6
8
10
12
14
16
18
20
TCMTB Conc. (ug/L)
96-h LC50
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*
*

SUMMARY

DEVELOPMENT OF AN APPROACH FOR ASSESSING TOXICITY OF AGRICULTURAL PESTICIDES TO RESIDENT AQUATIC BIOTA

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A multi-year program to assess the potential toxic effects of agricultural in-use pesticides to aquatic biota within the Lower Fraser Valley, B.C., was initiated by Environment Canada in 1988. The objective of Phase I, presented here, was to develop a site-specific approach for monitoring the potential toxic effects of pesticides from surface water runoff to resident aquatic biota. An initial review of the relative frequency of application of different herbicides and insecticides registered for use and marketed to farmers in the Lower Fraser River Valley was undertaken. Based on this appraisal, dinoseb (a herbicide) and endosulfan (an insecticide) were selected for detailed investigation including limited sediment and water sampling to determine baseline concentrations. A computer data base literature search was carried out to identify the environmental fate and biological effect of these two pesticides and their lethal and sublethal, acute and chronic, toxic effects.

Four sampling locations along the Nicomekl River in Southwestern British Columbia were selected for sampling of river water, surface water runoff, and sediments to obtain existing background concentrations of dinoseb and endosulfan. Three of the sites received farmland runoff via farm drainage/irrigation ditches which drained lands to the north and south of the river. At each site, samples were collected within each drainage ditch 10 m from the outflow location, and within the river 50 m above and below the drainage discharge point. The fourth site on the river, located approximately 10 km upstream, received no known farmland drainage, and served as a reference site.

In March 1989, prior to seasonal application of pesticides, samples of water and sediment were collected and analyzed for residual pesticide (dinoseb and endosulfan I and II) concentrations using solvent extractions followed by Florisil column chromatography and electron-capture gas chromatography. Appropriate quality control was included as part of the analyses. For surface water samples (ditch and river), concentrations of dinoseb and endosulfan in sediments were below the limits of detection of 10 ppb (dry weight). Ditch sediments showed the highest

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*Present address: Beak Associates Consulting Ltd., # 100 - 6846 King George Highway, Surrey, B.C. V3W 4Z9 concentrations of dinoseb (49 mg / dry kg), and endosulfan (428 mg / dry kg) recorded. The relevance of these findings was evaluated and discussed with respect to the study area, published findings for other areas, available water quality criteria for these pesticides, and the known water quality of the Nicomekl River.

Based on the information obtained, an approach for an aquatic toxicity monitoring program was developed. Emphasis was placed on the site-specific nature of the area, and the toxicity tests required. A sequential (tiered) approach was emphasized to allow for incorporation of new information at a later date, and to maintain flexibility within the overall program. The concept of using a battery of single-species toxicity tests (lethal and sublethal; acute and chronic) was followed in recommending specific test protocols and species. Specifically, biossays of ditch and river waters recommended included several series of acute lethal/sublethal toxicity tests using rainbow trout (Oncorhynchus mykiss) to determine effect/no effect concentrations. Chronic toxicity tests of some water samples using Ceriodaphnia sp., and fathead minnows (Pimephales promelas) were also recommended, as were tests with algal species (eg. Selenastrum sp.). The initial emphasis was placed on laboratory test methods. Field toxicity tests and surveys for biological effects were proposed for future study. Chemical analysis of those test waters shown to be toxic were included in the design. The specific sampling locations and monitoring frequency recommended were designed to address questions concerning the degree of toxicity for different runoff sources, seasonal variability, the amount of dilution required to achieve no-effect levels, and the extent to which individual pesticides would be identified as the sources of toxicity observed.

ACKNOWLEDGEMENTS

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We wish to acknowledge the extensive assistance provided by Drs. Carl Alleyne and Jim McKinley of the British Columbia Research Corporation who performed the pesticide analyses and provided interpretive advice. Ms. Angela Wong and supporting librarian staff within B.C. Research Corp. provided the computer-assisted search and retrieval of technical documents.

IMPACT OF ATRAZINE-BEARING AGRICULTURAL TILE DRAINAGE ON PLANKTON OF A NATURAL STREAM.

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Preliminary results are reported from a co-operative study between Agriculture Canada and Environment Canada on environmental impacts of atrazine as measured on a field scale. Tile drained plots of known area, flow and pesticide use were used as agricultural sources. A maximum tile drainage concentration for atrazine of 13.9 μg/L was reported while the maximum stream concentration was observed to be 1.89 μg/L. Phytoplankton and zooplankton samples were collected on a bi-monthly basis during the growing season. The study indicated possible atrazine impacts on phytoplankton at low concentrations under field conditions, when the natural stream flows were reduced resulting in lower dilution capacity. A 20 m section of the stream was affected by the tile drainage waters as measured by the resident biological community. Both atrazine and ambient environmental conditions are felt to be responsible for the observable results.

SUMMARY

Atrazine (2 chloro-4-ethylamino-6-isopropylamino-S-triazine) is widely used in Canada and United States as a selective pre- and post-emergent herbicide on many crops. Atrazine contamination of both surface and ground waters has been well documented in the literature. The present work examined the effect of atrazine-bearing agricultural tile drainage effluents on plankton of a small stream draining into salt spring Brook near Cornhill, New Brunswick, Canada.

Water and plankton samples were collected from eleven sampling stations, at approximately fortnightly intervals, at each site between June 9th and November 16th, 1989. Major ion, nutrient and atrazine analysis were carried out by the Water Quality Branch Laboratory, Environment Canada, Moncton. A quality control program was also employed to ensure the validity of the data.

Atrazine concentrations in the stream samples varied from 0.001 to 0.034 μg/L. The probable influence of ground water on the study waters was indicated by the presence of high sulfate and calcium concentrations in the water samples.

The plankton community examined include 103 species of phytoplankton and 10 species of zooplankton. The phytoplankton included diatoms (76), green algae (18), flagellates (5) and bluegreens (5). The most common phytoplankton species were Cocconeis placentula, Cyclotella meneghiniana, Fragilaria crotonensis, F. virescens, Melosira granulata, M. varians, Navicula pupula, Pinnularia apendiculata, P. borealis, Surirella linearis, S. ovulum, S. tenera, Synedra nana, S. ulna, Tabellaria fenestrata, T. flocculosa, Spirogyra sp., and Trachelomonas dybowskii. The zooplankton species were infrequent.

Response by the plankton to the atrazine concentrations (0.001 to 0.34 μg/L) was found to be minimal under field conditions when the natural stream flows were reduced resulting in reductions in dilution and/or self-purification of the stream. We recommend further prolonged field studies for more understanding of the ecological implications on the field streams in the presence of atrazine carrying agricultural effluents.

A COMPARISON OF SOME APPLICATIONS OF THE SEVEN-DAY *CERIODAPHNIA DUBIA* (CRUSTACEA:CLADOCERA) CHRONIC BIOASSAY.

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The seven-day *Ceriodaphnia dubia* chronic bioassay has been suggested as a potential regulatory or monitoring tool in Canada. This paper summarizes our experience with the test in three different applications, testing of pulp mill effluent, testing of uranium mine effluents and an ambient river water quality bioassessment. The pulp mill and uranium effluent tests showed increasing toxicity with increasing effluent concentration. However, in the uranium tests, growth and survival of the *Ceriodaphnia* in uranium test controls was low because the receiving water at northern Saskatchewan uranium mines is extremely dilute. Test results on ambient water, taken from 13 stations on the South Saskatchewan River, were not correlated with any chemical environmental variables. These studies show that reliance on any one set of tests for screening biological effects is problematical. Characteristics of the receiving environment and the appropriateness of laboratory species as surrogates for the most critical/sensitive species in the environment must be considered, although this implies complex monitoring and regulations.

A RAPID, HIGH-RESOLUTION DATA ACQUISITION SYSTEM FOR RECORDING AND ANALYZING PREFERENCE-AVOIDANCE MOVEMENTS.

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Preference-avoidance responses have become one of the more frequently investigated aspects of whole-animal sublethal effects, though test procedures are not yet standardized. Due to methodological problems, few studies have gone beyond measuring ratios of fish accumulating in pure versus contaminated test areas, or percentages of time spent there. Until recently, quantifying specific aspects of swimming behaviour as measures of response required tedious, time-consuming measurements from videotapes or chart recorders. By combining a previously described tracking device with an analog-to-digital converter, a microcomputer and specifically developed software, we can track and automatically record the linear position coordinates of a moving animal. The animal is tracked manually with a gunsight-type viewer connected to a potentiometer, which produces voltages (with spatial resolution in the millimeter range) corresponding to the animal's position. These voltages are continuously digitized by an A/D converter at a rate of 7.5 readings per second, equivalent to 133 ms per conversion. A BASIC program "captures" readings at user-selected rates; each reading is then stored along with the count number and elapsed time. When studying fish movements, we found it sufficient to sample movement paths at 1 s intervals. Our prototype worked adequately with an inexpensive Commodore 64C and a single floppy disk drive. Presently, we are using an IBM compatible 386SX PC, with a hard drive.

Once an efficient method of digitizing a fish's movements over time was developed, it became much easier to quantify many aspects of their movements. While the percent-time criterion has been the standard measure of response to contact with contaminants, it is an integrative measure of underlying behavioural variables which contribute to a measured "avoidance" or "preference" reaction. We use a SAS program to calculate not only percent-time spent on each side, but underlying elements such as trip durations, depth of penetration, swimming velocity and the ratio of swimming speeds entering and exiting a region of a tank. From these five elements of behaviour a total of 27 parameters are derived as possible indicators of response. Other measures could be quantified from these digitized data, such as total distance travelled or turning frequencies; this requires only small changes to the SAS program. Now that quantifying these other parameters is simple to do, it is feasible to compare the sensitivities of many parameters as response indicators, and not simply relying on the standard percent-time criterion. Such comprehensive comparisons are generally lacking in the literature. We are looking now into a formerly black box, as it were. While the percent-time measure is very easy to obtain and often useful, narcosis or chemokinetic reactions may result in incorrect interpretation of "time-spent" ratios as avoidance or preference. We have successfully applied our system to measure the preference-avoidance responses of lake whitefish (Coregonus clupeaformis) to contact with cadmium in a counter-current type trough (McNicol and Scherer, in press). Several behavioural parameters, including depth of penetration and swimming velocity were as sensitive or nearly so as the percent-time measure in indicating reactions to concentrations as low as 0.2 μg/L. Results from this study served to emphasize the importance of quantifing several behavioural measures of response in preference-avoidance studies. With our system, such quantification can now be accomplished simply and inexpensively.

A COMPUTERIZED MODEL FOR EVALUATING THE ADDITIVE TOXICITY OF MIXTURES OF METALS IN MINING EFFLUENTS AND RECEIVING WATERS

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ABSTRACT

A model will be presented for assessing the additive toxicity of mixtures of heavy metals in mining effluents. It can also be used to evaluate what ambient metal concentrations should be in order to achieve a predetermined toxicity downstream. This estimate, when used with the dilutions available, will help regulatory agencies set water quality objectives. Also, it can help determine what quality of effluent must be achieved in order to protect aquatic life in the receiving environment.

INTRODUCTION

Regulatory agencies issue permits which specify the level of metal contaminants that are allowed to be discharged from mining operations to receiving waters. We need tools to help evaluate if limits specified in effluent permits are protecting the environment. Among these tools are models which help understand conditions in the real world and estimate what the downstream effects of various metal mixtures might be. The USEPA has a variety of these models (Ambrose and Barnwell, 1989).

In this paper I describe a model that was first introduced by the Great Lakes Science Advisory Board (1981), and later modified by Alderdice & McLean (1982). This model could be a useful tool for evaluating the additive toxicity of metal mixtures in mining effluents. The model was computerized to make it more user friendly. The program can do the model calculations and all the user needs to do is supply the inputs. The model uses macro's in the Lotus 123 software to run "if" statements and do the calculations. A copy of the model is available from the author on 5¼ inch floppy disc.

Mining effluents from mills and effluent treatment plants are discharged under permit from storage facilities such as tailings or polishing ponds. They are complex mixtures consisting of

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metals, suspended solids, lime induced hardness, sulphate, sometimes nutrients, ammonia and frequently cyanide. They discharge to surface waters often with low dilution. These can be small headwater streams high in mountainous terrain. The receiving waters always support some form of aquatic life; some support fish, particularly in their lower reaches. Many of the streams support valuable salmonoid fisheries, including salmon, steelhead, cutthroat trout, and Dolly Varden char.

The pre-discharge or background water quality in mountain receiving waters is highly variable; often total metals are at or above water quality criteria or guidelines, particularly during freshet periods.

A brief discussion of water quality criteria and guidelines is important here. In British Columbia the provincial government refers to these as criteria (Pommen, 1989), the federal government calls them guidelines (CCREM, 1987). Essentially they mean the same thing. They are the "safe" level for a specified water use, such as protecting aquatic life. "Safe" means no effect after an infinite period of exposure to a given metal. The criteria are based on single species bioassays and toxicity tests using single contaminants, not mixtures of contaminants. The water quality criteria for metals are numbers which signify the threshold effect level in the aquatic environment. Below the criteria, aquatic organisms are "safe" (as defined). Above the criteria, there begins subtle toxic effects; a threshold has been crossed for the aquatic organism most sensitive to the specific metal.

The toxicity of mixtures is not well understood. Some work has been done in this area, but on simple mixtures such as copper, zinc and cadmium (Finlayson and Verrue, 1982; Roch, et.al. 1982, Cusimano, et.al. 1986). The complex mixture of metals and other contaminants in mining effluents has not been studied. It is known that mixtures can have additive, antagonistic and synergistic toxicities. Sublethal effect start at much lower concentration of metals than is necessary to induce an acute response. Sub-lethal effects on aquatic life are as important as acute. This is because mining effluents persist for many years while the mill is in operation. Many years of sub-lethal effect can add up to the same result as an acutely toxic effect over the short term.

When examining the toxicity of mining effluent mixtures, it is important that background water quality be recognized as an extant mixture of metals to which aquatic organisms are already exposed. The effluent added to the background water is additional to potentially high background numbers. Managers need an enhanced ability to asses the possible toxicity of an increase in the metals mixture in receiving waters over and above background, particularly under circumstances where the background already exceeds criteria to protect aquatic life.

The Great Lake Science Advisory Board, (1981), proposed a model in which the sum of the ratio of ambient concentrations of metals, divided by their respective water quality criteria, should equal or be less than 1.0 (Figure 1).

Figure 1.

$$\Sigma \frac{[\text{metal}]\ n}{\text{criterion}} \leq 1.0$$

Where: [metal] is ambient metal concentration.
Denominator is the published aquatic life criterion of that metal.
n = metals in mixture.

Alderdice & McLean, (1982), modified this model so that the sum of the ratios of the concentration of ambient metals divided by their respective maximum acceptable tolerance concentration (MATC) for a given fish species should equal or be less than 1.0. (Figure 2).

Figure 2.

$$\Sigma \frac{[\text{metal}]}{\text{MATC}} \leq 1.0$$

Where: MATC = maximum allowable tolerance concentration for a species

The main difference between the two is the use of criteria verses MATC. In Alderdice and McLean (1982), the equation used the LC_{50} of copper and zinc to Chinook salmon divided by an application factor (safety factor) to estimate the MATC. In the GLSAB (1981) model, the water quality criterion for each metal is assumed to be equal to the MATC. The equation in Figure 2 was the one computerized to simplify its use. Henceforth in this paper, reference to a model refers to the computerized version. The model essentially sums the ratios of each metal concentration to its criterion level.

A sum of ratios of 1.0 or less means the mixture has no effect, including no sub-lethal effect, over an infinite exposure period. If the sum of the ratios for the mixture is greater than 1.0, it is predicted to have some sub-lethal toxic effect.

Key assumptions are made to support this model. It must be assumed that the maximum acceptable tolerance concentration, or MATC, for each metal is the same as its criterion, adjusted as necessary for hardness or pH. This assumption is valid if what is being protected is the most sensitive species in the aquatic system, not just fish. The criteria used in the computerized model reported here are approved and working criteria for BC (Pommen, 1989), or, where no B.C. criteria are established, the CCREM guidelines (1987), are used.

If it is assumed that the MATC equals the criteria, provided that the sum of ratios is 1.0 or less, the mixture will protect all aquatic life even the most sensitive species. An important question is if a mixture contains metals all at their criterion concentration, is the mixture "safe" as defined. Indications are that it is not, as reported by Wong, et. al. (1978), who show significant losses in phytoplankton in Hamilton Harbour under these conditions.

If MATC's cannot be equivalent to the criteria, what good are water quality criteria? What are they for if not to signify a threshold effect level for the most sensitive aquatic species?
The model uses the assumption that toxicities are additive only. No provision is made for antagonistic or synergistic mechanisms, or partial additivity within the metals mixture. The model uses the assumption that the metals have a similar toxic effect (but are not equally toxic) and that toxicity is linear down to the no-effect level. Also, the assumption is made that after the mixture in the effluent enters the receiving water, there is no immediate natural detoxification in the environment.

The computerized version of this model automatically calculates the MATC (criterion) for each metal from the corresponding average hardness and pH input data where this is entered by the user. To date the model has been developed to work with: cadmium, copper, zinc, lead, arsenic, aluminum, silver and nickel. It sums the ratios for all, or any combination of these metals. The model also provides for the back calculation of target ambient metal concentrations from a preselected toxicity objective or sum of ratios.

The model may be used with either total or dissolved metals. The use of total metals is likely to over-estimate the mixture toxicity. The use of dissolved metals is likely to under-estimate the mixture toxicity, given the assumptions listed above. Most water quality criteria are published as total metal.

Tables 1 through 3 illustrate examples of the use of the model. Table 1 shows a sum of ratios for water quality in Upper Foxy Creek, upstream from a treated effluent from Equity Silver for 1989.

The average hardness is 50mg/l. Using 5 metals, the sum of ratios is 2.23. This is already over 1.0. How should this be interpreted? This is attempted below, but first examine the ratios in Table 1. Total aluminum with a ratio of 1.25 is the single largest contributor to the sum. This indicates that managing to restrict further additions of aluminum to Foxy Creek would be an objective. Table 2 shows the sum of ratios for average values of selected metals in the Bulkley River at Quick, using data collected between 1982 and 1988.

Table 1

UPPER FOXY WATER QUALITY, 1989.
(Total metals)

avg. hardness = 50 mg/l

	Total Metals	Measured (ug/l)	MATC (ug/l)	Ratio
	Cd	0.2	0.66	0.3
	Cu	2.9	6.7	0.43
	Zn	5.0	30.0	0.17
	As	3.5	50.0	0.07
*	Al(d)	125.0	100.0	1.25
			Total	2.23

* see text.

In Table 2 the sum of ratios is 6.65, which is much higher that 1.0. Each of lead, arsenic, copper and cadmium all exceed 1.0, only zinc and aluminum are below 1.0. Note that the BC criterion for aluminum is for dissolved metal.

Table 2
BULKLEY RIVER AT QUICK, 1982 -1988.

avg. hardness = 24.8 mg/l.

Total Metals	Measured (ug/l)	MATC (ug/l)		Ratio
Cd	0.5	0.38		1.3
Cu	5.1	4.3		1.2
Zn	8.0	30.0		0.27
As	69.0	50.0		1.4
* Al(d)	48.0	100.0		.48
Pb	28.0	13.8		2.0
			Total	6.65

*see text

Using total metals, the Bulkley River is on average higher than the BCMOE criteria for Cd, Cu, As, and Pb. This would likely be an over-estimate of the bio-available metal in the river. The metals are probably connected to high sediment loads during freshet. More to the point, what does a sum of ratios of 6.65 mean in terms of the overall long term toxicity of the Bulkley River? Is the Bulkley River with this mixture of background metals safe (as defined) to aquatic life?

The model for the Bulkley River at Quick was re-run using the average dissolved metals collected over the five year period. In this case, the sum of ratios is reduced to 5.48 (Table 3.).

Table 3

BULKLEY RIVER AT QUICK, 1982 - 1988

avg. hardness = 24.8 mg/l.

Dissolved Metal	Measured (ug/l)	MATC (ug/l)	Ratio
Cd	0.3	0.38	0.79
Cu	3.3	4.3	0.76
Zn	6.0	30.0	0.2
As	64.0	50.0	1.3
Al	48.0	100.0	0.48
Pb	27.0	13.8	1.95
		Total	5.48

In this example the contribution dissolved aluminum makes to a sum of ratios drops well below 1.0 leaving lead and arsenic as contributing most to the final result. Lead in particular appears to be a concern in the Bulkley River at Quick. Using dissolved metals probably under-estimates the additive toxicity of the mixture.

It is still not entirely clear how to interpret a value of 6.65 or 5.48. One interpretation is that the existing mixture of contaminants in the Bulkley River is exerting a subtle toxic effect on aquatic life. This conclusion is supported by ratios exceeding 1.0 for individual metals such as lead and arsenic. Remember that the "safe" level sum is 1.0 or less, and "safe" is defined as no toxic effect, including no sub-lethal effect, over an infinite exposure period.

What the additive toxicity model shows for the Bulkley River at Quick is that the background mixture of metals in the river already has some subtle toxic effect on aquatic life. This interpretation has implications for regulatory agencies if new contributions of contaminant from metal mines are proposed for the river. It is to this end that a provision was made within the model to back calculate ambient concentrations given a preselected level of toxicity or sum of ratios.

The model back calculates to answer the following questions: How much additional metal mixture can be tolerated in the receiving water, if any? How can the model be used to license better effluent discharge limits?

The user can specify a target sum of ratios and give ambient average hardness and pH input data. The model will calculate the mixture that is necessary to achieve that sum of ratios. The toxicities are proportioned on the basis of the toxicity of silver. This is because the criterion level for silver, at 0.1 ug/l, is the lowest of the metals used in the model.

A mine discharge will combine with existing metal contaminants in the background to make a new mixture of contaminants downstream (Figure 4). Downstream concentration after mixing can be estimated using a simple dilution mass balance equation.

Figure 4

The background mixture may already be a problem. As we have seen illustrated in the Foxy Creek and Bulkley River examples, the background mixture may already exceed 1.0. The back-calculation provision of the model helps if the user first preselects a sum of ratios. The model determines the concentrations of metals in the mixture to achieve the sum. Deducting the background concentrations, and using the worst case dilution available, the target concentrations of metals in a mining effluent can be determined.

For example, the Bulkley River at Quick has an existing sum of ratios of 6.65 based on average values. If the management objective is to keep the increase in metals contamination below a 25% average increase, a new sum of ratios would be about 13. The model will back-calculate what the individual metal concentrations should be in the river to arrive at a sum of ratios of 13. Using the average background concentrations of metals in the river, along with known minimum or worst case dilutions available, the necessary effluent mixture can be estimated.

Permits and licences from regulatory agencies are supposed to contain limits which protect the environment. Regulators need to improve their ability to determine the proper level to permit. The models that are available to asses the additive toxicity of mixtures will help to refine the information available to do this. Managers have to find ways of implementing the new knowledge that is developed by the science of toxicology. We will be looking increasingly to toxicologists for guidance in managing water quality. A better insight is required into the toxicity of mixtures and hopefully this need will be responded to by the research community. Further, there is a necessity to use real field data to calibrate models such as the one presented here.

Acknowledgement

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STREAM PERIPHYTON AND BENTHIC INSECT RESPONSES TO ADDITIONS OF TREATED ACID MINE DRAINAGE IN A CONTINUOUS-FLOW ON-SITE MESOCOSM

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ABSTRACT

An on-site continuous-flow trough mesocosm was used to examine changes in the composition and abundance of periphytic algae and benthic invertebrates from additions of a 10% solution of treated acid mine drainage (AMD). Five control and five treatment troughs supported an assemblage of periphyton and invertebrates that colonized from water withdrawn from Foxy Creek a stream that receives treated AMD from the Equity Silver mine, central B.C. Canada. A water intake for the mesocosm was located upstream of the AMD discharge. The treated AMD was delivered to the apparatus through a pipeline laid in a canal that carried the AMD to Foxy Creek. After three weeks of colonization in the troughs, additions of one part AMD to 10 parts flow-through water was delivered to the treatment troughs and continued for three weeks. Analyses of variance of measurements of abundance and biomass indices contained high power values and indicated that the null hypothesis that AMD addition did not significantly change the algal and insect composition and abundance could not be rejected. Advantages and disadvantages of the mesocosm with regards to the relative sensitivity of the measured parameters for use in examining effects of the AMD additions are discussed. Conclusions were that quantitative on-site experiments using the mesocosm apparatus is a powerful approach for use in setting guidelines for the discharge mainly because of its capability of integrating ecosystem processes in experiments where hypothesis testing is possible.

INTRODUCTION

Acid mine drainage (AMD) is a widespread environmental problem that has been examined extensively in the literature. Most reports of effects of AMD on aquatic biota are laboratory bioassays of single taxa (eg. Daniels *et al.* 1979). Field studies are less common and deal mainly with monitoring temporal changes in the chemical milieu and biological community in response to AMD additions (Dills and Rogers 1974, Hoehn and Sizemore 1977, Short et al. 1990). Only a few studies have used experimental approachs to examine *in situ* effects of AMD (eg. Peckarsky and Cook 1981). Largely because of logistic constraints (ie. examining *in situ* effects after an AMD discharge has occurred) most of these field studies are upstream - downstream comparisons of water quality indices; a design that lacks the necessary controls to infer causality.

Despite the abundance of these data, predictions of stream ecosystem responses to AMD additions have been crude at best, given that complex interactions among system components and the variety of chemicals (Schindler 1987) cannot be resolved in most single organism bioassays or monitoring studies. The general inability to extrapolate findings from the laboratory to the field leaves water quality managers with considerable uncertainty in setting allowable dilution rates of AMD that is discharged into streams. The general lack of information to indicate sublethal effects and to predict food-web or geochemical interactions (eg. Schindler et al. 1985) demands site-specific, *in situ* experiments that allow testing of explicit hypotheses at the community or ecosystem level.

A continuous-flow, on site, mesocosm apparatus was used to test the null hypothesis that the dilution of 10 parts water to 1 part treated AMD does not affect the composition and abundance of periphytic algae or benthic macroinvertebrates in Foxy Creek, a stream that receives discharge of treated AMD from the Equity Silver Mine located in north central British Columbia, Canada. The 10:1 dilution ratio was established by regulatory agencies but is presently not supported with technical rationale to show that the dilution is adequate to protect downstream ecosystems.

The use of a stream mesocosm has many advantages over other techniques used for testing toxic effects of AMD. Streams function as open systems and the flow-through design of a trough having suitable substrata to support a representative biotic assemblage provides for exchange of components necessary in geochemical dynamics, behavioural interactions of organisms, food web interactions, etc. Processes are integrated in a trough mesocosm, as they are in a natural stream, thus allowing for extrapolation of findings to natural ecosystems (Gearing 1989). As stream water continuously flows through the troughs, system processes can be allowed to equilibrate and then a perturbation from this central state can be measured. The ability to replicate experimental units in a mesocosm also provides for a true experimental approach with appropriate controls for the testing of hypotheses, which is generally not possible in other field monitoring studies. The power of significance tests (Cohen 1988, Peterman 1990) can be determined to provide estimates of the ability of the tests to detect real differences. Uncertainty is then removed in the process of setting regulations for AMD. Considering these advantages, it is not surprising that experiments using mesocosms have been increasingly used to examine a variety of toxicants including acidification (Allard and Moreau 1987), pesticides (Muirhead-Thomson, 1987), and well as for examining basic ecosystem processes (Mundie et al. 1983).

Disadvantages to mesocosm studies relate to constraints on realism including the lack of structural heterogeneity in streams, lack of normal variation in rates of discharge, and the short-term nature of such studies. Many of these variables, however, can be manipulated in mesocosm designs to make the system as simple or as complex as is required. An important factor is that the fate of organisms emigrating from the mesocosm is unknown. Although the fate of animals may be important in toxicity tests of individual taxa (in which case a mesocosm would likely not be used), the actual fate of animals was not important in this study. Whether animals emigrated from the troughs as a behavioural response to additions of AMD or whether they died from the AMD was irrelevant. Any selective dissappearance of taxa from a mesocosm receiving AMD was considered evidence of an impact of the AMD additions.

Throughout this study, the treated AMD (raw AMD that is limed to increase the pH from 2.2 to about 8) is considered a single chemical entity despite its well known complexity. There was little point in examining the toxicity of individual constituents of the AMD when interactions of chemicals and speciation of chemicals is difficult or impossible to predict (eg Campbell and Stokes, 1985). Also, the toxicity or sublethal effects are unlikely to be linearly related to the concentration of any one chemical constituent. Most important, is that the experiment was designed as an on-site test of system responses to routine AMD discharge.

MATERIALS AND METHODS

The experiment was conducted at the confluence of Foxy Creek and a drainage canal that carried treated AMD from the Equity mine site (Figure 1). This location allowed a pipeline to be laid upstream in Foxy Creek to supply water to the mesocosm and it facilitated a second pipeline to be laid in the canal to supply the treated AMD.

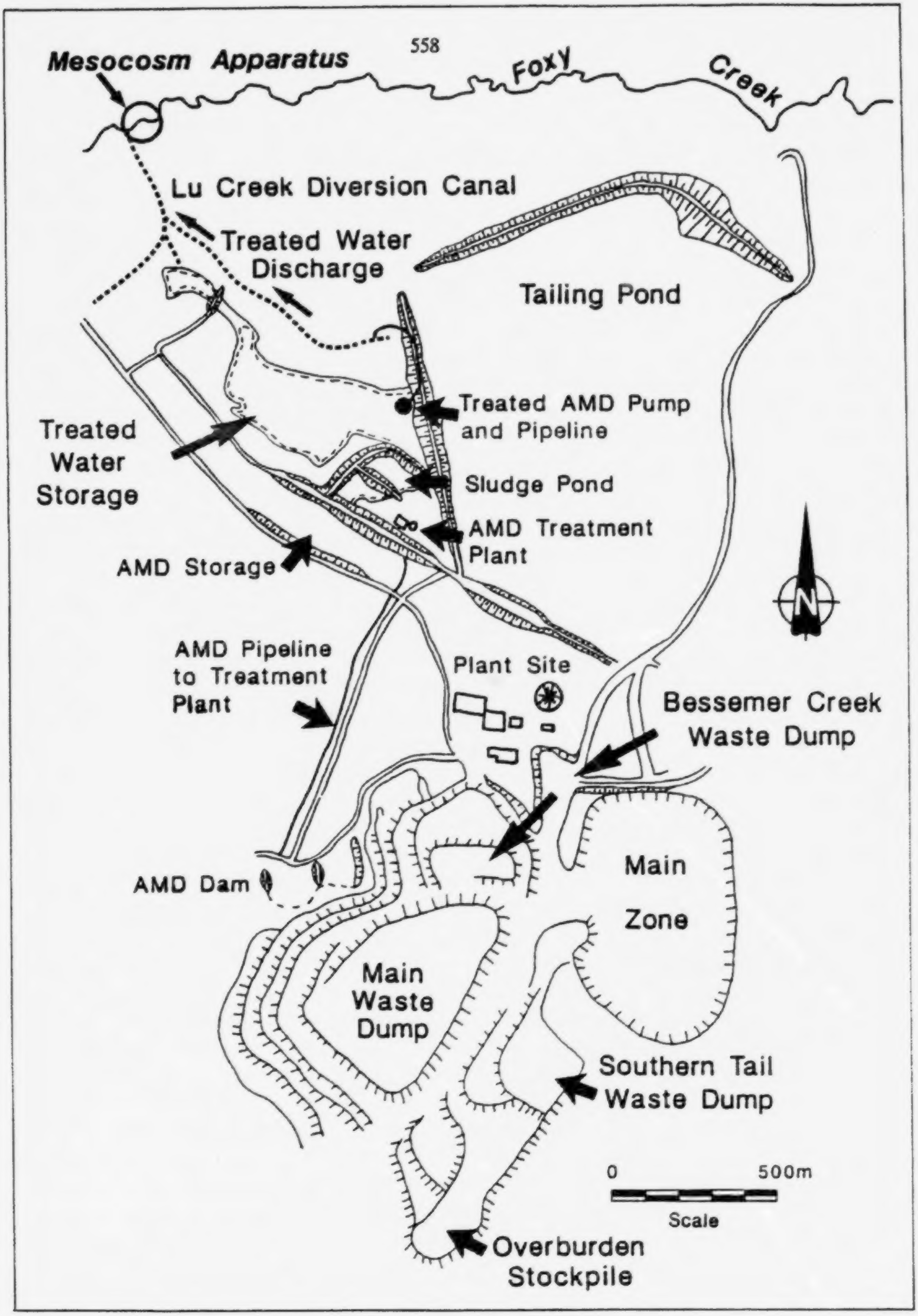
Mesocosm Apparatus
Foxy Creek
Lu Creek Diversion Canal
Treated Water Discharge
Tailing Pond
Treated AMD Pump and Pipeline
Treated Water Storage
Sludge Pond
AMD Treatment Plant
AMD Storage
N
AMD Pipeline to Treatment Plant
Plant Site
Bessemer Creek Waste Dump
AMD Dam
Main Zone
Main Waste Dump
Southern Tail Waste Dump
0
500m
Scale
Overburden Stockpile

Figure 1. Layout of the Equity Silver Mine and location of the experimental trough apparatus.

The mesocosm consisted of ten flow-through troughs (each 1.52 m long x 0.2 m high x 0.2 m wide) that were fabricated with 1/4 inch clear plexiglass and assembled on a series of joists laid over top of the stream channel. The design followed that used by Mundie et al. (in prep). Briefly, water and biota carried in suspension in Foxy Creek were supplied to the mesocosm via gravity through a 100m (6 inch diameter) pipeline installed in Foxy Creek. The pipeline was fitted to a head tank and water was delivered to each trough through a standpipe assembly. The standpipe for each trough could be slightly rotated to maintain water flow at $0.4\ \mathrm{L \cdot s^{-1}}$ without the use of valves. The treated AMD was delivered via gravity through the second 260 m (2 inch diameter) pipeline that was laid in the Lu Creek diversion canal (Figure 1) which carried the treated AMD from the water treatment facilities at the mine. A series of gate valves on the trough apparatus controlled the flow of AMD to the troughs to maintain a 10:1 flow dilution ratio, the same ratio which was maintained between Foxy Creek and discharge of the treated AMD.

A mixing chamber consisting of an angled baffle that created turbulence in the water and AMD supply was located at the head of each trough. Downstream of the mixing chamber was a 1.2m section within which 3/4 inch drain rock was laid to a depth of five cm. Downstream of the gravel was a 0.32 m section that was fitted with a sheet of open cell styrofoam DB (Snowfoam Products Ltd. El Monte, California) that provided a surface for the sampling of periphyton biomass. The gravel and styrofoam sections were separated with a 7 cm high baffle which produced a water depth of 7 cm over the gravel. At the flow rate of $0.4\ \mathrm{L \cdot s^{-1}}$, the surface current velocity was $6\ \mathrm{cm \cdot s^{-1}}$. Water leaving the gravel section flowed over the baffle and dropped onto the styrofoam surface creating turbulence at its upstream end. The water then flowed in a laminar pattern over the remaining styrofoam from which samples were collected.

Water flow through all 10 troughs began on July 23, 1989. Three weeks were allowed for colonization of the troughs by aquatic insects. The troughs were randomly assigned to serve as controls or treatments (AMD addition). The flow of AMD to the five treatment troughs began on August 13, 1989. At that time, the collection of emerging insects began by placing a plexiglass emergence trap over top of each trough. The traps were identical to those designed and used by Mundie et al. (in prep.). Sizing was adjusted to seal all openings on the top opening of the troughs, thus preventing the airborne escape of emerging insects. The mesocosm was shut down with the termination of water and AMD flow on September 8, 1989.

Water samples were collected from the outflow of each trough on eight occasions for analysis of dissolved metals and macronutrients. Samples were filtered in the field and analyzed for total alkalinity (acid neutralizing capacity), specific conductance, pH, sulfate, nitrate plus nitrite, soluble reactive phosphorus (SRP), and a suite of metals. Laboratory procedures followed APHA (1985). Metals were determined by inductively coupled plasma techniques. Low level detection of copper and aluminum was measured by graphite furnace methods.

Samples of periphyton to measure biomass accumulation were taken according to the accrual technique reported by Perrin et al. (1987). Briefly, the biomass attached to, or settled on the styrofoam substrata in each trough was collected weekly by removing a core with the open end of a light-tight 7 dram plastic vial. All samples were frozen in the field at -15°C and shipped on dry ice to laboratory facilities within three weeks. All samples were analyzed for chlorophyll *a* concentrations after extraction in 90% acetone using the fluorometric methods outlined in APHA (1985). Change in biomass was followed through two time series (July 24 through August 10 and August 14 through September 7). At the end of each time series, an additional core was extracted from each trough, and preserved in Lugol's solution for later taxonomic examination according to methods outlined in Northcote et al. (1975).

Drift of aquatic insects from each trough was sampled over three hours and 24 hours one day prior, and one day following AMD additions. An additional 24 h collection was made on August 30 to examine drift rates after an extended period of AMD addition. Drift samples were collected in 3 gal. plastic pails fitted with 253 μm mesh screen on an outlet opening. The pails were hung at the downstream ends of the troughs and continuously filtered all water to the 253 μm size fraction during the sampling period. Emergence of adult insects from the troughs were collected continuously in the emergence traps. The traps were emptied weekly and the insects were preserved in 5% formalin. Benthos samples were collected at the end of the experiment (September 8) by removing the entire contents of each trough.

In the laboratory, the insect samples were washed through a series of nitex sieves (1.0, 0.47, 0.1 mm mesh). Insects retained on the 1.0 and 0.47 mm sieves were preserved in 70% ethanol for further sorting. The remaining size fraction was preserved for future reference. Benthic insects were separated from the detritus and algae using a dissecting microscope at 6.4x magnification for fractions retained on the 1.0 mm sieve, and 16x magnification for the fraction retained on the 0.47 mm sieve. Sorted samples were preserved in 70% ethanol for counting and identification. Identification of the insects from drift, emergence, and benthos samples were made using keys in Merritt and Cummins (1984), Cole (1969), Edmunds et al. (1976), and Wiggins (1977). All counts were made at 6.4x and 16x magnification for the 1.0 and 0.47 mm size fractions respectively.

RESULTS

Physical and Chemical

Daily maximum and minimum water temperatures in the trough apparatus ranged from 14°C and 7.5°C respectively in late July to a peak of 16.0°C and 10.9°C respectively in early August. Thereafter, temperatures declined to a daily maximum of 6.3°C and a minimum of 5.6°C in the first week of September.

Concentrations of boron, barium, cadmium, chromium, molybdenum, and vanadium were always less than the detection limit of 0.01 $mg \cdot L^{-1}$. Cobalt and lead concentrations were always less than 0.1 $mg \cdot L^{-1}$ and nitrate plus nitrite-N concentrations were consistently less than 0.02 $mg \cdot L^{-1}$. Nickel was always less than 0.05 $mg \cdot L^{-1}$.

Data for the remaining chemical variables are summarized in Table 1 for the period before and after the start of treated AMD additions. At the 10% dilution rate, the addition of treated AMD increased the conductivity by 7.5 times over the control. This increase was mainly attributed to a 54-fold increase in sulphate concentrations, a 9.6-fold increase in calcium levels and a 5.1-fold increase in magnesium concentrations. Concentrations of manganese and zinc increased only slightly due to the AMD additions. Concentrations of copper increased marginally to reach 0.002 $mg \cdot L^{-1}$ in the treatment troughs. Aluminum concentrations did not change after the start of the AMD addition. SRP concentrations also did not change but were more than 7 times higher than levels found in other nearby streams. They are also high relative to the inorganic N concentrations (nitrate plus nitrite) and are in a range that would indicate that growth of the periphyton community was limited by nitrogen, based on experiments conducted in another river of the north central region (Perrin, unpubl. data).

Periphyton

Samples of periphyton collected from the styrofoam were dominated by diatoms (Table 2). *Hannaea sp.*, *Diatoma sp.*, and *Synedra sp.* were the most common genera. Chlorophytes including *Ulothrix sp.*, *Closterium sp.*, *Mougeotia sp.* and *Cosmarium sp.* represented less than 2.5% of the periphyton community. Trace numbers of the blue green alga, *Anabaena sp.* were also found. There were no major changes in the composition of algae collected from control and treatment troughs over the 25 days of AMD addition.

Table 1: Chemical concentrations at full mixing of treated AMD and Foxy Creek water before and after additions of treated AMD. Data are means of 30 samples collected throughout the experiment. Data are means, (standard error), and sample size from samples collected at the outflow of the troughs. Units are mg·L^{-1}.

Measure	Before AMD			After Start of AMD Additions					
				Control			Treatment		
pH	7.36	(0.11)	30	7.44	(0.16)	25	7.42	(0.20)	25
Conductivity	47.2	(0.21)	20	51.8	(0.20)	5	386.0	(11.2)	5
Alkalinity	25.0	(0.35)	30	24.1	(0.54)	25	26.7	(0.67)	25
SO_4	1.37	(0.05)	30	2.53	(0.27)	25	137.9	(7.08)	25
Ca	5.19	(0.06)	30	5.06	(0.02)	25	48.5	(1.30)	25
Fe	0.05	(0.002)	30	0.087	(0.007)	25	0.078	(0.005)	25
Mg	2.04	(0.01)	30	2.12	(0.01)	25	10.83	(0.28)	25
Mn	0.01	(0.00)	30	0.01	(0.00)	25	0.06	(0.002)	24
Zn	0.01	(0.00)	30	0.01	(0.00)	25	0.011	(0.0006)	24
Al	0.122	(0.068)	2	0.117	(0.02)	6	0.121	(0.026)	5
Cu	<0.001	(<0.001)	2	<0.001	(<0.001)	6	0.002	(<0.001)	5
SRP	0.01	(<0.001)	30	0.009	(<0.001)	25	0.007	(<0.001)	24

Table 2. Comparison of the taxonomic composition of periphyton from control and treatment troughs, before (August 13) and after (September 7) the addition of treated AMD to the treatment troughs.

Taxon	Controls (%)		Treatment (%)	
	13 August	7 September	13 August	7 September
Diatoms	97.4	97.5	98.6	98.7
Hannaea	61.1	64.6	67.0	60.9
Diatoma	11.4	9.8	11.8	13.8
Synedra	15.0	14.3	9.0	6.7
others	7.5	8.8	10.7	17.3
Chlorophyta	2.3	2.4	1.2	1.3
Cyanophyta	0.3	0.1	0.2	0.0

A repeated-measures, nested ANOVA (trough nested in treatment) of the chlorophyll a concentrations before the AMD was added indicated no difference in biomass levels between control and treatment troughs ($p > 0.2$) through time, thus confirming that the randomized allocation of controls and treatments did not produce differences in biomass as an artifact of the trough layout. Areal biomass increased exponentially, reaching a mean peak biomass (PB) of 1.1 $\mu g \cdot cm^{-2}$ over the 18 days.

After clean substrata were installed and the AMD addition was started, periphyton biomass was consistently greater in the treatment troughs compared to the controls over the 25 day time series (Figure 2). Another repeated-measures, nested ANOVA showed that the differences were highly significant ($p < 0.0005$). In the troughs receiving the AMD additions, PB reached 1.3 $\mu g \cdot cm^{-2}$ after 22 days compared to a peak of 1.02 $\mu g \cdot cm^{-2}$ over the same time period in the controls.

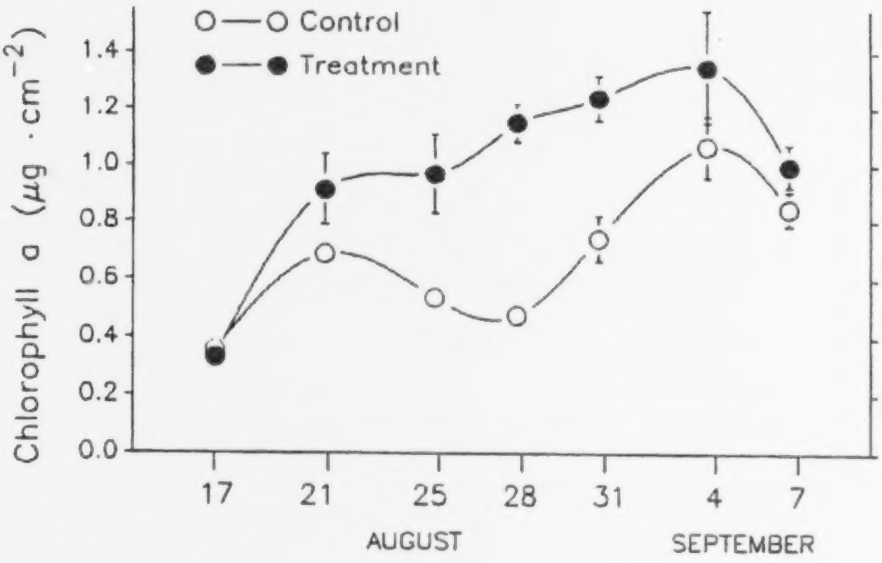

Figure 2. Time course changes in mean chlorophyll *a* concentrations (±1 SE) on styrofoam substrata in control and treatment troughs after the initiation of AMD additions (August 14 through September 7). The apparent absence of error bars on some data points indicates that they were too small to extend beyond the symbols indicating the means.

Macroinvertebrates

At the end of the experiment an average of 2213 and 2468 animals were found in the benthos of each of the control and treatment troughs respectively. This total number was represented by about 20 taxa (Table 3). Baetid mayflies dominated the community. Chironomids of the Orthocladiinae were next most common, followed in importance by *Ameletus* sp., *Zapada* sp., Chloroperlidae, Tanypodinae, and Tanytarsini, each of which were present in similar numbers.

"Taxonomic richness" (Krebs 1978) is used as an index of diversity in this paper. It is essentially a measure of community structure and is simply the number of taxonomic units per sample. Compared to more complex diversity indices based on information theory (ie. the Shannon-Wiener function; see Krebs 1978) that can be difficult to interpret and are often misused, richness is easy to interpret. Taxonomic units used in this study are genera except for some identifications which proceeded only to the family level (Table 3 and 4).

Taxonomic richness of the drift was about 55% of that of the benthos (compare Table 3 and 4), a finding that was expected since numbers of animals in the drift were less than 5% of the benthic population. Most benthic animals will eventually occur in drift but the proportions of common taxa in benthos and in drift are usually very different. Numbers of Baetids dominated the drift on all sampling dates. They were followed in importance by Acari, *Zapada* sp., and *Micrasema* sp. in the mid-August samples. In the August 30 samples, the Baetids numerically represented more than 85% of the community. *Zapada* sp. and chironomids of the Orthocladiinae followed in importance.

Treatment Effects on Benthos

Benthos samples were analyzed using ANOVA with treatment as the main factor. The results were summarized by total abundance, taxonomic richness, and abundance of individual taxa (Table 3).

Total densities were about 12% higher in treatment troughs relative to the control troughs, but these differences were not significant ($p>0.07$). The power of the test was 0.80. A total of 18 taxonomic groups were examined separately for treatment effects but none were significantly different at a critical alpha of 0.003, the significance level applied after the Bonferroni correction for multiple comparisons (Rice 1989). This correction accounts for the possibility of significant random differences in multiple comparisons. Two genera of mayflies (*Rhithrogena* sp. and *Paraleptophlebia* sp.) had differences at the $0.05>p>0.03$ but this was not significant when the Bonferroni correction was applied. The relative differences in numbers of these genera between the control and treatment were also not consistent: numbers of *Rhithrogena* sp. were greater in the treatment troughs compared to the controls but the opposite was the case for numbers of *Paraleptophlebia* sp.

Taxonomic richness did not differ significantly between the treatment and control troughs ($p>0.68$).

Treatment Effects on Drift

The rates of drift before and after the initiation of AMD flow were tested using a three-way ANOVA, with treatment, date (before or after AMD addition) and sampling period (3 or 24 h period) being the main factors. The interaction term for treatment x date was the measure of interest since a significant interaction would indicate that the rates of drift changed disproportionately from the treatment and control troughs across dates. Ten taxonomic groupings had sufficient numbers for analysis (listed in Table 4). Data were transformed to logarithms of $x+1$ prior to analysis. For both the 3 hour and 24 hour samples, none of the ten groupings had significant treatment x date interactions (all with $p>0.18$) (Table 4;24 hour data, Figure 3; 3 and 24 hour data). The critical level of alpha was 0.005, determined after the application of Bonferonni's correction. The power of the comparison of drift rates between treatments on the day after the start of AMD flow was >0.95.

Table 3: Mean numbers and taxonomic richness (±1 SE) of benthos collected from 5 replicate control and treatment troughs on September 8. The critical alpha level for comparisons of the numbers of animals of each taxa between the control and treatment troughs was 0.003 after applying the Bonferroni correction for multiple comparisons (i.e., 0.05/number of comparisons [=17]): see text for explanation.

Grouping	Control		Treatment		Probability of Effect
Total	2213.0	(120.5)	2468.0	(30.5)	>0.07
Taxonomic Richness	20.6	(0.68)	20.2	(0.74)	>0.68
Ephemeroptera					
Baetis	863.4	(83.8)	1090.8	(101.0)	>0.14
Ameletus	170.0	(12.7)	180.4	(27.4)	>0.8
Cinygma	48.4	(11.1)	73.8	(7.3)	>0.14
Rhithrogena	21.0	(3.3)	34.0	(3.9)	>0.03
Epeorus	3.0	(2.5)	1.6	(0.5)	>0.9
Paraleptophlebia	17.2	(2.1)	10.4	(1.7)	>0.03
Ephemerellidae	12.8	(1.8)	11.8	(1.5)	>0.7
Plecoptera					
Zapada	143.6	(14.7)	130.4	(9.5)	>0.5
Chloroperlidae	137.4	(9.9)	155.2	(21.9)	>0.5
Doroneuria	16.0	(1.5)	22.6	(4.5)	>0.15
Isoperla	12.4	(3.2)	16.2	(4.8)	>0.6
Trichoptera					
Micrasema	13.4	(5.1)	22.2	(11.3)	>0.5
others	3.4	(0.25)	3.0	(0.8)	>0.45
Diptera (Chironomidae)					
Tanypodinae	196.0	(21.6)	203.8	(18.6)	>0.75
Orthocladiinae	405.0	(21.6)	394.8	(25.1)	>0.7
Tanytarsini	146.2	(17.7)	112.2	(12.7)	>0.15

Table 4: Composition of drift collected over 24 hour periods before (August 12-13 sample) and after (August 13-14 and August 30 samples) AMD additions. Data are mean numbers ±1 SE. The probability of a treatment by date interaction, indicating a disproportionate change in drift rate in the Aug. 12-14 samples or an absolute difference between treatments and controls in the Aug. 30 samples is indicated in the third column.

Grouping	Control		Treatment		Probability of Effect
1: August 12-13 sample					
Total	92.6	(5.91)	97.0	(12.3)	
Richness	11.0	(0.84)	12.4	(0.40)	
Baetis	34.6	(3.71)	37.6	(14.4)	
Epeorus	0.0		0.4	(0.25)	
Serratella	1.0	(0.63)	0.6	(0.40)	
Zapada	12.0	(1.48)	13.6	(2.58)	
Micrasema	14.0	(3.19)	18.2	(3.69)	
Parapsyche	1.2	(0.97)	1.2	(0.37)	
Orthocladiinae	2.8	(0.37)	3.6	(0.81)	
Simuliidae	2.8	(0.86)	3.0	(1.09)	
Acari	15.6	(2.71)	10.6	(1.17)	
miscellaneous	8.6	(1.50)	8.2	(0.40)	
2: August 13-14 sample					P: (Treatment x Date)
Total	82.2	(10.28)	97.0	(6.67)	>0.40
Richness	10.8	(1.20)	10.2	(0.58)	>0.72
Baetis	30.2	(3.81)	41.2	(7.48)	>0.18
Epeorus	0.0		0.4	(0.25)	>0.35
Serratella	0.6	(0.40)	0.8	(0.20)	>0.50
Zapada	10.2	(1.85)	15.8	(4.60)	>0.98
Micrasema	9.4	(2.09)	10.0	(3.27)	>0.94
Parapsyche	0.4	(0.25)	1.0	(0.63)	>0.71
Orthocladiinae	4.0	(0.84)	2.0	(0.45)	>0.23
Simuliidae	1.6	(0.68)	0.4	(0.25)	>0.78
Acari	17.4	(2.38)	20.6	(1.54)	>0.56
miscellaneous	8.4	(2.42)	4.8	(0.97)	>0.51

/Continued

Table 4, Continued

3: August 30 sample					P: (Control < > Treatment)
Total	132.8	(18.69)	99.6	(6.69)	>0.13
Richness	8.6	(0.75)	6.4	(0.81)	>0.08
Baetis	114.0	(18.2)	89.2	(4.87)	>0.22
Epeorus	0.0		0.2	(0.20)	
Serratella	0.2	(0.20)	0.2	(0.20)	
Zapada	5.8	(2.82)	2.6	(0.60)	>0.29
Micrasema	0.0		0.0		
Parapsyche	0.0		0.0		
Orthocladiinae	3.6	(0.40)	2.2	(0.58)	>0.08
Simuliidae	0.6	(0.25)	0.2	(0.20)	
Acari	0.0		0.0		
miscellaneous	8.6	(1.03)	5.0	(2.00)	>0.14

The emergence of aquatic insects was generally variable and the total numbers were low (Table 5). The most commonly collected taxa were midges of the subfamily Orthocladiinae. The only other taxa of significance were the Baetids and the Ceratopogonids which were captured occasionally. On the last sampling date, numbers of *Tanytarsini* increased and exceeded those of the Orthocladiinae. *Simulium* sp. also became more important on the last two sampling dates.

In more than 70% of the samples, non-aquatic species of the Hymenoptera, Collembola, Homoptera, and of the fly families Muscidae and Scatophagidae were found. These collections were not included in calculations of total numbers or taxonomic richness in Table 5. Although the Dolichopodid flies may be considered terrestrial, they are common in lotic margins. Since they have at least a semiaquatic life history, they were included in Table 5.

Several taxa found in the emergence traps were never encountered in either the benthos or drift, likely because they were too small to be retained on mesh sizes used to seive the samples and also because of specific habitat preferences. The very small sizes of the Ceratopogonidae and *Simulium* sp. likely passed the 0.4 mm sieve and hence may have been missed. Because the counts of *Cinygmula* sp., Limnophelidae, Empididae, and the Coleoptera were only occassional and irregular, it is unlikely that they had become established in the troughs and may simply have entered the trough apparatus as they were emerging or drifting near the water intake. The Dolichopodidae have terrestrial larval stages are would not be expected to become established in the benthic community. The Ephydridae are lentic brine or shore flies that may have originated from the many ponds that are close to Foxy Creek and particularly the holding ponds that are near the mine tailings area. Similarly, the Chaoboridae are lake dwellers and would not be expected to become established in the benthic community of the troughs.

Table 5. Mean numbers and taxonomic richness (±1 SE) of emergence collected from 5 replicate control (C) and treatment (T) traps on four dates after the initiation of AMD additions.

Grouping	August 18		August 26		Sept. 1		Sept. 8	
	C	T	C	T	C	T	C	T
Total	6.8	8.2	15.8	12.0	21.0	18.6	28.2	12.8
	(3.1)	(5.2)	(8.5)	(5.3)	(7.5)	(7.3)	(14.7)	(8.0)
Richness	3.2	2.8	4.4	3.0	4.8	5.8	4.0	3.4
	(1.3)	(2.0)	(0.9)	(1.0)	(1.6)	(1.1)	(0.7)	(0.9)
Ephemeroptera								
Ameletus sp.	0.2	0.2	0.2	0			0.2	0
Baetis sp.	0	0.6	0.2	1	0.2	0.4	0.2	0.2
Cinygmula sp.					0.2	1.4		
Tricoptera								
Limnephilidae	0.2	0						
Coleoptera								
Elmidae	0.2	0						
Unknown			0.2	0	0	0.6		
Diptera								
Dolichopodidae	0.8	0.8	1.6	1.6	0.6	1	0	0.6
Ephydridae	0.2	0.2	1	0.2	0.4	0.4	0.2	0
Chaoboridae			0	0.2	0.2	0		
Ceratopogonidae	0.2	0.2	2	0.8	1	0.2	0.4	0.4
Orthocladiinae	4.4	6.0	9	8.2	14.2	11.4	7.8	5.2
Tanytarsini	0.4	0	1	0	3.4	2	16.8	12.2
Simulium sp.			0.6	0	0.6	1	1.8	0.2
Empididae					0.2	0.2		

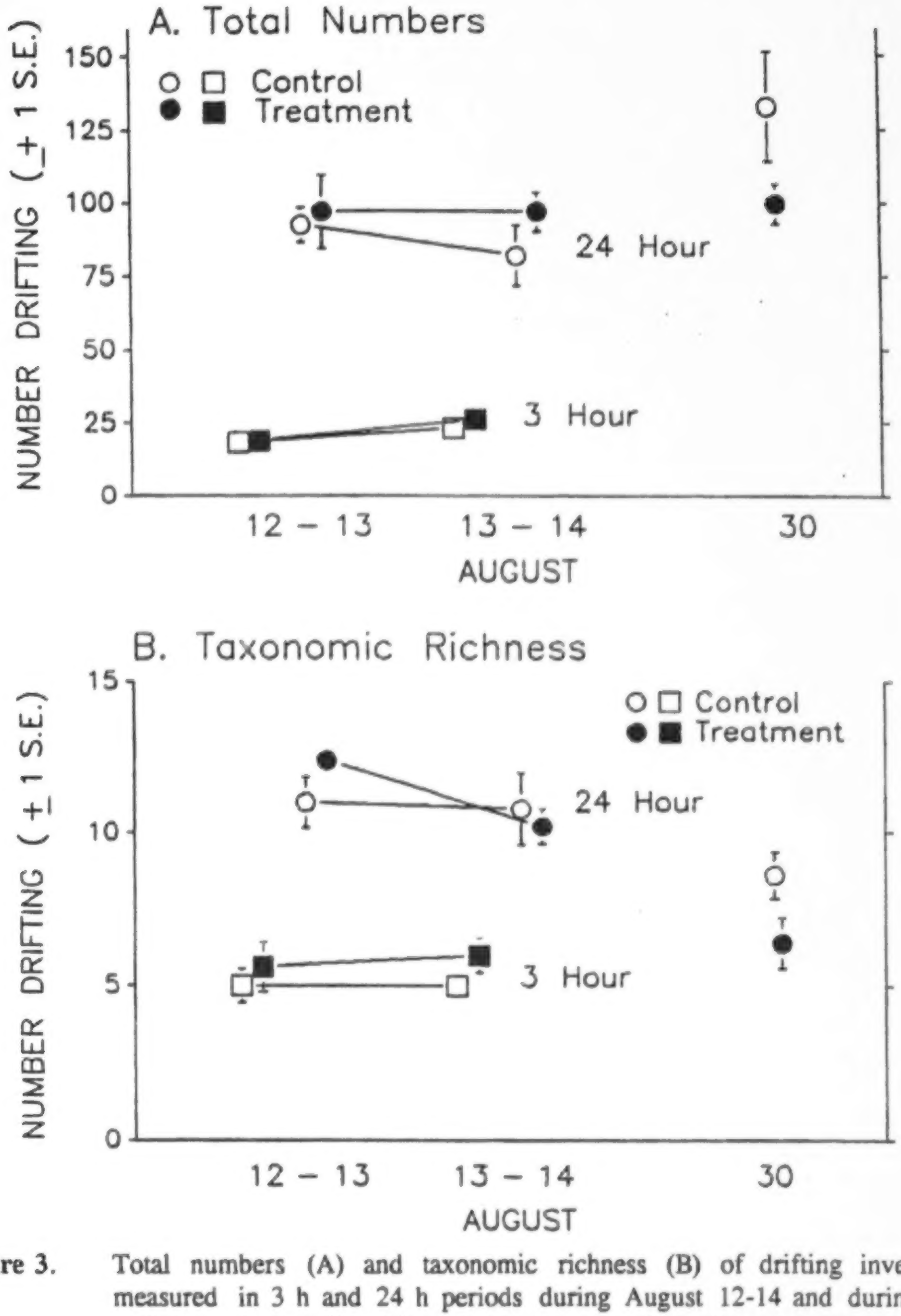

Figure 3. Total numbers (A) and taxonomic richness (B) of drifting invertebrates measured in 3 h and 24 h periods during August 12-14 and during a 24 h period on August 30. Lines drawn between data show the treatment x date interactions, none of which were significant. See text for significance values. AMD additions were initiated on August 13.

The 24 h drift samples from August 30 were also analyzed with ANOVA and no taxonomic group revealed any treatment effect (all with $p > 0.08$, Table 4). The critical level of alpha after the Bonferonni correction was 0.008. A similar finding applied to the total number of insects drifting ($p > 0.13$) and for taxonomic richness ($p > 0.08$).

Neither taxonomic richness nor total numbers in the drift differed significantly across treatments for any drift sampling period (Figure 3). Both indices of samples from the treatment troughs appeared slightly higher for the 12-14 August period, but those from control troughs appeared greater on August 30. These differences were not significant (Table 4).

Treatment Effects on Emergence

An ANOVA of logarithmically transformed emergence data with treatment and date as main effects indicated no significant effect of treatment ($p > 0.35$), while there were differences among dates ($p < 0.001$). Emergence increased in a linear pattern that was independent of the addition of the treated AMD. Total abundance was less than 10 animals per week when the experiment started but increased to 20-30 animals per week at the end. The greatest emergence occurred during the week of lowest temperatures (maximum daily temperature ranged from 6.3 to 10.8°Cand the minima ranged from 5.6 to 5.9°C).

DISCUSSION

The addition of treated AMD from the Equity Mine had no significant negative effect on any of the biological measures examined in the experiment at the prescribed 10:1 dilution rate. The only change that the treated AMD addition had on the stream mesocosm was a significant increase in areal biomass of periphyton. Apparently the treated AMD did not produce a toxic effect but rather an enhancement of algal biomass accrual. Since the treated AMD contained levels of several micronutrients, it is possible that any micronutrient deficiency was alleviated by the chemical additions. Although a treatment effect was apparent, it is important that the differences in biomass were very small and well within the range that can typically be found in a natural stream. The analyses of invertebrate abundance suggest that at the dilution rate of 10 parts Foxy Creek water to 1 part treated AMD that has chemical concentrations similar to those measured in this study, the discharge of treated AMD will not impact on the abundance of macroinvertebrates downstream of the Lu Creek diversion canal in Foxy Creek. Since macroinvertebrates are the primary size fraction of animals that are consumed by salmonids (Irvine and Northcote, 1983), this finding indicates no impact on the abundance of fish food organisms by the addition of treated AMD over a three week period.

The use of troughs as stream mesocosms in this study provided a powerful experimental approach for assessing the effect of additions of treated AMD on ecosystem processes at the Equity Mine site. This finding suggests that replicated, *in situ* stream mesocosms offer the experimental testing of explicit hypotheses while providing a high degree of realism by incorporating most of the natural ecosystem processes (eg. Mundie et al. in prep.). The realism provided by such an approach made possible the strong inference of the effects of treated AMD additions on the Foxy Creek system. The troughs successfully supported periphyton and aquatic insect communities and the various ANOVA's which were used as the basic tool for examining the impact of AMD additions had power values in excess of 0.80. Peterman (1990) has suggested that in general, power values ≥0.8 are necessary for results of significance tests to be conclusive. By obtaining high power values in the drift and benthos analyses, we have confidence that the experimental and equipment design used in this study was suitable for producing conclusive evidence that the null hypothesis of no effect of the added AMD could not be rejected.

Although several measures of invertebrate response to the AMD additions were examined, it is worth noting that with the present mesocosm design, the most convincing data on responses came from changes in numbers in the benthos. Very subtle treatment effects in the drift may be difficult to detect in small troughs because drift leaving all troughs is a combination of what is coming in from the stream and passing through the troughs, and what is being produced by processes in the troughs themselves. The trough drift may be far exceeded by stream drift thus masking treatment effects. Hence, drift measurements are useful mainly when treatments produce large changes in numbers of animals that leave the troughs relative to numbers drifting through the troughs. In our study, drift measurements were used to examine an immediate "behavioural" response to the onset of AMD additions. Our null hypothesis was that any change in the chemical milieu would not lead to a large exodus from the benthic community and the results were highly conclusive in not rejecting this hypothesis. The emergence data were considered relatively imprecise largely because of the small numbers of animals that were collected. Total numbers were about 10 times lower than those found in identical emergence traps used by Mundie et al (in prep.) in a mesocosm established in Carnation Creek, a small coastal stream in British Columbia. The main difference between experiments was that in the present study, surface current velocities were double those used at Carnation Creek. Many more animals would be expected to be lost in water outflow from our apparatus during the process of emergence. Benthos measurements, by comparison, were not confounded by flow-through dynamics in the troughs and thus were more sensitive to treatment effects than the other measurements.

An advantage of mesocosm experiments is that they can be run for any period of time, and in so doing, can be used to examine time-related changes in ecosystem dynamics. In comparison to conventional toxicity bioassays that last no more than a few days in a laboratory, the present experiment may be considered to be a chronic test. Lab bioassays, however, deal only with one or a few organisms and interactions within and between species assemblages are ignored. The present trough experiment, by contrast, involved a minimum of three weeks for an assemblage of periphytic algae and benthic insects to become established and the toxicity test dealt with a combination of acute effects on individual organisms plus changes in the integrated structure and function of the trough assemblage. At sublethal levels of an added toxicant, changes in these latter processes may not be detectable for days or weeks after the toxicant is added. In the present study, a three week period of AMD addition was arbitrarily selected as a reasonable time to detect changes in the benthic community. It is well accepted, however, that sublethal effects may require longer periods to be noticable in the mesocosm and thus in an actual stream ecosystem. It is known, for example, that very small, early stages of insects are more sensitive to additions of toxicants than are larger animals (Gauss et al. 1985, Allard and Moreau 1987) and where this is important, numbers of larger animals may not change for an extended period of time in relation to reduced recruitment by younger larval stages. Although early instars of many taxa were enumerated in our benthos samples, it is unknown what proportion of the smallest animals were lost by sorting with a 0.47 mm sieve. We did recover large animals that undoubtedly represented the majority of biomass and the fraction that is most likely to be consumed by salmonids. But, the potential importance of smallest size classes in determining populations in the longer term, suggests that the experiment be considered an acute test of effects of the AMD additions. A longer term experiment or complete enumeration of smallest animals would be required to confirm longer term changes in the structure and function of Foxy Creek.

An important assumption of this study is that results from the mesocosm can be extrapolated to Foxy Creek. It is understood that the lack of structural heterogeneity in the mesocosm may produce an assemblage of organisms that is not identical to that in the stream. Taxonomic richness approximated 20 taxa which is probably less than that in Foxy Creek. But, the mesocosm did support an interacting assemblage that was derived from Foxy Creek and thus could be considered representative for examining reductions in numbers of animals in an interactive system from the treated AMD additions.

The ability to extrapolate findings from a mesocosm to a stream is important from a regulatory perspective. Agencies that are involved in permitting waste discharges use published criteria (eg. CCREM water quality guidelines for Canada, 1987) as an overall guideline. These data give receiving water criteria for individual metals, but information on effects of levels of mixtures of metals is notably lacking. Consequently, effluent permits which must identify critical levels of metal mixtures do so without support of technical rationale. The value of the present experiment is the ability to extrapolate findings of effects of treated AMD on a representative assemblage of organisms to determine potential changes that may occur in Foxy Creek. The present findings indicate that the discharge of treated AMD in a dilution ratio of 10:1 to Foxy Creek will not produce short term changes in the abundance of benthic insects that are available for consumption by salmonids. The data suggest that present permit regulations in effect at the Equity Mine are adequate for short term protection of the benthic community in Foxy Creek.

The high statistical power of the approach suggests that it could reduce uncertainty in the permitting process at other sites and for other toxicants. Past uses of mesocosms in ecotoxicological studies has been limited by the failure to replicate, and thereby the determination of statistical power to draw inferences has not been possible (eg. Cooper and Stout 1985, Servos and Mackie 1986, Allard and Moreau 1987). Careful consideration of replication in the experimental design used in this study is evidence that statistical power can be applied to the development of water quality criteria by reducing uncertainty in experimental data.

While the design of this experiment was simple, more elaborate experiments incorporating a graded series of chemical additions or factorial designs are possible. The potential for defining threshold effects, and considering taxon-specific or process-specific impairment at different rates of treatment allows for a highly accurate measure of the effects of chemical manipulations. An incorporation of a gradation of treatments to define threshold effects is likely to be most useful. By calculating a graded response curve, a measure of the minimum level of dilution of the treated AMD milieu that causes an impact on ecosystem processes could be identified.

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PLATFORM SESSION

Histopathological Responses

Chair: M. Wolfe

HISTOPATHOLOGY OF CLAMS LIVING IN DEPOSITED MINE-TAILINGS: SO WHAT IF CLAMS HAVE LESIONS.

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Axinopsida serricata (Bivalvia: Thyasiridae) is a numerically dominant clam in coastal waters of British Columbia subjected to natural or anthropogenic disturbance. While examination of lesions in natural populations of certain bottom fish has gained acceptance as a tool for monitoring environmental contamination, histopathology of molluscs has not been widely accepted. This is, in part, because of a lack of information on normal form and function, and because of a perceived variability in tissue structure unrelated to the disturbance of concern. In order to investigate the potential of histological lesions in *A. serricata* as a monitoring tool, field populations were sampled in Holberg Inlet and Quatsino Sound, British Columbia from benthos affected by the discharge of copper-mine tailings, and from a reference site in Mill Bay, Saanich Inlet.

Based on a quantitative analysis of the digestive gland, gill, kidney, gonad and stomach, the relationship between lesions and site, size, season, sex and parasitism were explored. Within the digestive diverticula, changes in height of digestive cells, increased incidence of vacuolation, and necrosis were observed in clams collected nearest the tailings outfall. Lesions of the ctenidia included swelling and hyperactivity of ctenidial mucocytes, oedema of lateral cells, loss of frontal cilia, and some disorganization of ctenidial filaments. Large extracellular nephroliths in the kidney and accumulation of tertiary lysosomes in a specialized area of stomach epithelium in clams from lower Holberg Inlet and Granby Bay were possibly an indication of increased metal uptake.

Univariate statistical analyses of lesions quantified by morphometrics and stereology indicated significant between-sample differences for 12 of 13 quantified putative lesions. In most cases, the pattern of differences between samples was related to proximity to the mine-tailings discharge, seasonally-dependent maturation of gonads, or both. Based on a principle components analysis of six of the tissue variables, those clams collected from three stations in Lower Holberg Inlet which were in closer proximity to the tailings discharge pipe were more severely affected than clams collected from the reference site, upper Holberg Inlet, and Quatsino Sound. The first three principal axes captured greater than 60% of the variability of the original data set. The first principal axis was related to time of collection (just before spawning or when clams were recovering) while the second axis was related to proximity to the discharge pipe.

Most lesions were relatively independent of clam size (and thus of age and duration of exposure), sex, or ectoparasitism by a flagellate. In spite of the notorious natural plasticity of molluscan tissues, the variability can be partitioned to provide an effective interpretation of exposure to stressors.

THE ONCOFISH: *Xiphophorus* HEREDITARY MELANOMA AND IMPLICATIONS FOR CARCINOGENICITY TEST MODEL

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Astract - An *in vivo* animal model, heritable melanoma in platyfish/swordtail hybrids, is described. The model documents multiple genetic factors mediating tumorigenesis. Distinct genotypes can be experimentally produced that are resistant or highly susceptible to carcinogenic substances. This oncofish model lends itself well for testing pollutants, for example in our waters.

Introduction

Tumour formation is a multistep process resulting from changes in the complex, genetic network that guarantees controlled cell division and progression of cells into a differentiated state in which cells have lost their proliferative capacity. As a consequence of these genetic changes, cells fail to achieve or lose their differentiated state leading to expression of undifferentiated characteristics such as cell growth. Thus, cancer appears to be a particular form of genetic disease.

Immense insight into the kind of tumorigenic changes and into the molecular basis of tumour formation has been accomplished through the discoveries of oncogenes and anti-oncogenes, tumour suppressor genes. Oncogenes representing altered forms of the normal genes, proto-oncogenes, appeared to induce the tumour phenotype in a single-hit, genetically dominant fashion. However, the result that malignancy can be suppressed by fusing a malignant to a normal cell suggested that the tumour phenotype was due to loss of a function required to maintain the normal phenotype. This further suggested that the tumour phenotype arose as a consequence of a recessive mutation in a wildtype allele, i.e., in a tumour suppressor gene, whose homologue had already undergone a recessive mutation (Harris 1986). In agreement with this interpretation, segregants of these cells that had regained the tumour phenotype after a few generations showed complete loss of particular chromosomes. Thus, the normal, double (diploid) set of chromosomes is necessary to hinder carcinogenesis (see Harris and Watkins 1965; Klein et al. 1971; Stanbridge et al. 1982). Hemizygosity of a recessive mutation however, predisposes to cancers and mutation in the remaining wildtype allele leads to the tumour, such as retinoblastoma in humans.

As indicated, a number of lines of evidence suggest that tumourigenesis is a multistep process and thus, neither a single alteration in a proto-oncogene nor the two changes (hits) in the diploid tumour suppressor genes are sufficient to produce expression of the tumour phenotype. A cooperative effect of both gene classes has been suggested and some experimental evidence has been presented (for review, see Weinberg 1989).

Need for evaluation of environmental contaminants

It is obvious that our environment is contaminated with substances of carcinogenic potential. Because carcinogens can interfere with the mechanisms addressed above, most likely by mutating the differentiation-important genes, it is urgent to evaluate these substances in appropriate carcinogen test systems.

In vitro systems, while helpful in deciphering some of the genetic principles of tumorigenesis, have the distinct disadvantage of not reflecting the complex differentiation mechanisms present in an organism. Therefore, *in vivo* models must also be employed in order not only to fully understand the genetic mechanisms but also to recognize the full action of contaminants in tumorigenesis. Because genetic factors are the targets for carcinogenic substances, the system should have a defined genetic background tailored for easy identification of the effects of hazardous substances.

Genetically conditioned melanoma formation in small tropical fish - primarily a consequence of loss of a tumour suppressor gene

The *in vivo* model of hereditary melanomas in fishes of the genus *Xiphophorus*, commonly known as platyfish and swordtails, has been studied extensively to recognize the genetic determinants in tumorigenesis. These fish originally stem from Central America but have been kept in laboratories since the early twenties and are highly inbred. Some species exhibit polymorphic pigment cell patterns composed of a distinct melanin producing pigment cell, macromelanophore. The patterns are genetically determined by a series of partially sex-linked complex loci, *Sd*, *Sr*, *Sp*, etc. These loci comprise at least two genes, the macromelanophore gene, responsible for the cell type, and a pattern gene, directing the cells to distinct areas and thus giving rise to the pattern such as the spotted-dorsal (Sd) pattern, or the striped-side (Sr) pattern, i.e., spots in the dorsal fin or stripes on the flank of the fish.

While these patterns do not change in the parental strain their expression is changed in intra- and interspecifc hybrids. Certain patterns give rise to skin cancer, melanoma, in specific backcross generations according to Mendelian laws; such crosses are schematically depicted in the figure. A platyfish female homozygous for *Sd* when crossed with a swordtail gives rise to heterozygous F_1 hybrids that over-express the Sd-pattern, i.e., a Sd-pattern that covers the entire dorsal fin area. Backcrossing the F_1 to the swordtail yields backcross offspring that segregate according to Mendel 1:1, i.e., half the offspring inherit the *Sd* but form a dorsal fin melanoma instead of a Sd-pattern. Most importantly, within the melanoma segregants a 1:1 segregation is observed: half exhibit a slow-growing benign type melanoma while the other half exhibit a fast-growing, malignant melanoma. This 1:1 segregation suggests that the melanoma types are mediated by a single autosomal gene and can be proven by the segregation patterns of second backcross offspring; backcross hybrids carrying a benign melanoma again yield a 1:1 segregation of benign to malignant melanoma carriers and those with malignant melanomas yield only malignant melanoma carriers in the *Sd*-group after backcrossing with the swordtail.

Following the segregation patterns of chromosomes which are marked by strain specific isoenzymes in the backcross generation of melanoma carriers it has been clearly shown that the benign melanoma carriers are heterozygous, i.e., have a heterozygous platyfish/swordtail chromosomal constitution for chromosome V, and the malignant melanoma carriers have a homozygous swordtail constitution for the same chromosome. Further genetic analyses have localized the gene on the particular platyfish chromosome and combined morphological and biochemical studies have revealed that the gene is necessary for terminal differentiation of the pigment pattern cells and has therefore been termed *Diff* (Vielkind 1976). Thus, because tumorous growth results from the loss of differentiation function due to homozygous loss of the platyfish gene, the *Diff* must be considered a tumour suppressor gene (for details, see Vielkind et al. 1989).

Loss of the tumour suppressor gene is insufficient for melanoma formation - collaborative genetic factors

The association of the melanoma phenotype with the loss of the *Diff* tumour suppressor gene implies that this event is critical for this tumour formation. However, it is not a sufficient step since the F_1 hybrids, although hemizygous for the *Diff* tumour suppressor gene, do not form benign melanomas as the hemizygous backcross hybrids do. This suggests that other, additional, genetic events are necessary conferred by the overrepresentation of chromosomes of the swordtail which cannot supply the correct function for normal pigment cell differentiation. Furthermore, another gene has been identified that is located in or close to the *Sd* locus and can hinder melanoma formation in backcross hybrids despite the loss of *Diff*. It, however, allows tumour formation after being mutated. These results indicate that multiple genetic factors are involved in melanoma formation and point to the complexity of tumorigenesis (see Vielkind et al. 1989).

Carcinogen treatment of hemizygous backcross fish yields a variety of tumours - possibility for an in vivo test system and identification of genes necessary for normal cell differentiation

The studies on the melanoma formation in these fish have documented that multiple genetic disturbances are the underlying principle in tumour formation. Such a genetic situation is given in the backcross generation which represents a hemizygous situation for many chromosomes (genes). According to this principle, treatment with carcinogens should be expected to result in the induction of various kinds of tumours in the backcross fish (see Schwab et al. 1978a,b) but rarely in the F_1-hybrids and parental strains. The results of these experiments were clear cut and support the notion that an unstable genetic background, i.e., the possibility to induce loss of functional genes, is the reason for high susceptibilty for carcinogenesis. One particularly interesting group for carcinogen-induced mutational changes are the backcross fish that are hemizygous for *Diff*. Only one mutation is necessary to render this gene non-functional and thus will lead to melanoma formation. Following this rational these fish have been used to study the effect of UV-irradiation (Setlow et al. 1989). These results allow two conclusions. Firstly, these backcross fish represent a suitable and sensitive test system for the testing of potential carcinogenic substances due to the manipulated, labile genetic make-up. In other organisms, multiple genetic changes must accumulate, requiring presumably higher carcinogen concentrations and longer exposure times and also a higher number of animals to be treated. Secondly, on pure scientific grounds, this system allows identification of chromosomes, and so genes, which hinder the formation of specific tumours and are necessary for proper differentiation of the cell type of a given tumour.

Suitability and cost effectiveness of fish system

One female produces 50-100 offspring every 4 weeks and is fertile for two years. In general, 40-60 animals are kept in 10-15 gallon tanks, an aquarium facility of 75 square feet can hold 4,000-5,000 fish, food for this number of fish is $200-300. The maintenance of the fish stocks as well as breeding of the backcross fish requires one animal care taker. Thus, this system is not only by far more cost effective than other possible in vivo models (mice, rats) or even tissue culture systems, it is the most suitable for the testing of pollutants in our water systems.

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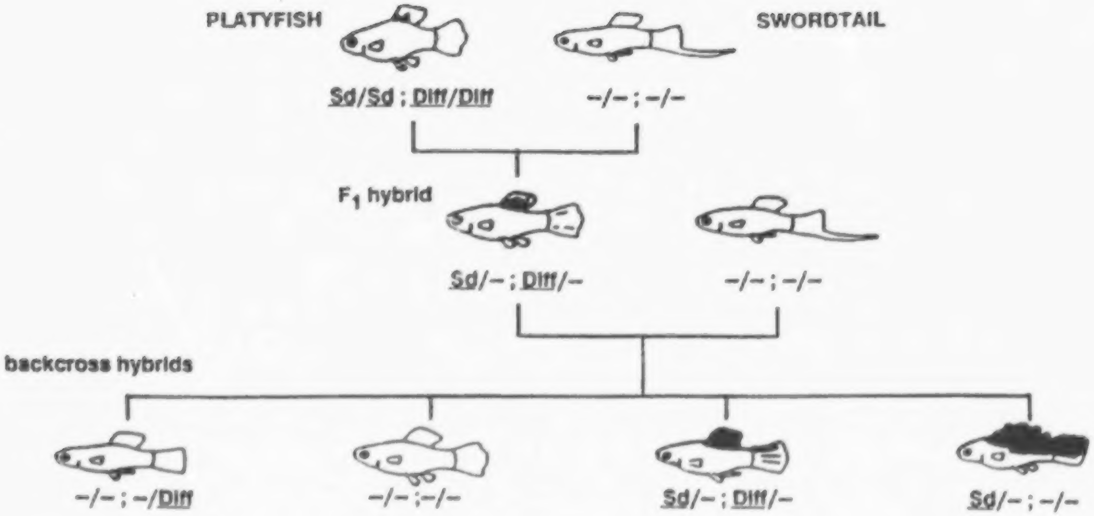
PLATYFISH
SWORDTAIL
Sd/Sd ; Diff/Diff
–/– ; –/–
F1 hybrid
Sd/– ; Diff/–
–/– ; –/–
backcross hybrids
–/– ; –/Diff
–/– ;–/–
Sd/– ; Diff/–
Sd/– ; –/–

Outline of crosses documenting principles of melanoma formation in *Xiphophorus* fish. A female platyfish that is homozygous for the X chromosome-linked pigment pattern locus *Sd* and the autosomal tumour suppressor gene *Diff* is crossed with a swordtail which does not carry equivalent genes on the homologous chromosomes: the F_1 female hemizygous for the genes mentioned is then backcrossed to a swordtail male. The Sd-pattern of the platyfish female is overexpressed in the F_1 and even more so in backcross hybrids leading to benign and malignant melanoma in the *Sd*-carrying group. Sex-reciprocal crosses have the same result. For further details see text.

HISTOPATHOLOGY OF MEDAKA (ORYZIAS LATIPES) USED AS A TEST ANIMAL IN BIOASSAYS OF NATURAL WATERS AND VARIOUS EFFLUENTS

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EXTENDED SUMMARY

The bioassay to test the carcinogenic potential of natural waterways, groundwaters or effluents is performed in a 24-foot mobile trailer equipped with five-gallon glass aquaria and solenoid-type proportional diluters that deliver different concentrations of test water in a flow-through system.

Medaka at 14 days of age are exposed to 10 mg/l diethylnitrosamine (DEN) in water in the laboratory for 48 hours. An equal number of 14-day-old medaka are not exposed to DEN. The Den-initiated and non-initiated fish are transferred to the biomonitoring trailer where they are exposed to various concentrations of test water. Control fish, initiated and not initiated with DEN, are housed in clean water in the trailer and in the laboratory.

At 13 weeks after initial exposure to the test water the medaka in each tank are divided into three groups. One-third are sacrificed. One-third remain in the biomonitoring trailer for an additional 13 weeks at which time they are sacrificed. One-third are returned to the laboratory to clean water for an additional 13 weeks at which time they are sacrificed.

The medaka are sacrificed and fixed in Bouin's solution after a small incision is made in the abdomen to facilitate fixative penetration of abdominal organs. Whole fish are processed in a Tissue-Tek™ automatic tissue processor, embedded in paraffin, and step sectioned in a sagittal plane to yield two left paramedian sections, two right paramedian sections and one mid-sagittal section. Thirty tissues are examined in each fish. These tissues are bone (vertebra), brain, chromaffin tissue, corpuscle of Stannius, esophagus, eye, gallbladder, gill, heart, hematopoietic tissue, interrenal tissue, intestine, kidney, liver, nares, ovary, pancreas, peripheral nerve, pineal organ, pituitary gland, pseudobranch, skeletal muscle, skin, spinal cord, spleen, stato-acoustic organ, swim bladder, testis, thymus, thyroid tissue and urinary bladder.

Results of the histopathologic examination for each medaka are entered into a computer and are tabulated. Summary tables are compiled so that lesion incidence in each treatment group can be compared. The pathologist writes a narrative summary and explanation of the findings.

The DEN-initiation protocol targets the liver as the organ most likely to respond to promotional effects of compounds in the test water. Although liver neoplasia is the anticipated outcome if promotional agents are present in the test water, any tissue in the fish potentially could be affected by toxic changes or neoplasia if the medaka are exposed to the appropriate compound.

Systematic recording of lesions that occur in control and test-water exposed medaka from study to study will result in the establishment of a data base of spontaneous and treatment-related lesions in medaka.

The U.S. Army Research Methods Branch is pursuing a comprehensive basic and applied research program to support the medaka bioassay.

TOXICOPATHIC HEPATIC LESIONS IN JUVENILES OF THREE SPECIES OF FLATFISH FROM PUGET SOUND, WA; RELATIONSHIP TO OTHER INDICATORS OF CONTAMINANT EXPOSURE.

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Liver neoplasms are rare in young wild fish; thus, one must consider other liver lesions as bioindicators of contaminant exposure effects in monitoring studies where target fish specimens are not adult. In addition, it has been argued that effects of contaminant exposure are more reliably assessed in juvenile fish which have not yet migrated extensively. We have addressed these issues by histologically examining juveniles of English and rock sole and starry flounder captured from nine sites in Puget Sound, and measuring fluorescent aromatic compounds (FACs) in bile and other biochemical indices of contaminant exposure. Although neoplastic and preneoplastic lesions were detected at low prevalences, much higher prevalences of several types of nonspecific and unique degenerative lesions were detected in all three species. These lesions have been experimentally induced in fish by exposure to various toxicants, and/or have been associated with contaminant exposure and the process of liver neoplasia in adult fish. Prevalences of these earlier bioindicators were significantly higher at the more contaminated sites compared to the less contaminated sites. Moreover, prevalences of these lesions in all three species were significantly correlated with mean bile FACs levels at the sites, in agreement with the results of previous studies utilizing adults. These findings further support the utility of certain liver lesions other than neoplasms as early indicators of biological damage in wild fish exposed to xenobiotics.

THE RELATIONSHIP BETWEEN LIVER LESIONS IN LAKE ONTARIO SLIMY SCULPINS AND OTHER INDICATORS OF HEALTH AT THE INDIVIDUAL AND POPULATION LEVEL. Fitzsimons, J.D. Fisheries and Oceans Canada, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, Ontario, L7R 4A6 (416-336-4862); R.W. Owens, United States Department of the Interior, Fish and Wildlife Service, Oswego Biological Station, Oswego, NY, 13126.

Lake Ontario, of all the Great Lakes is the most contaminated. Published data indicates the widespread movement of sediment bound heavy metals, chlorinated hydrocarbons and polynuclear hydrocarbons away from sources in the nearshore zone to the deeper offshore waters. The slimy sculpin, an obligate benthivore generally restricted to these deeper offshore waters shows evidence of clustering of liver lesions which are suggestive of a chemical etiology. While liver lesions on their own may not be lethal they may be associated with lesions that are lethal either in the short-term or in the long-term through effects on gonad development and ultimately population size. Based on 12 sites in Lake Ontario the relationship between liver lesions and other health indicators will be examined.

MOLTING, METAL METABOLISM, METALLOTHIONEINS IN BLUE CRAB AND LOBSTER.

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The discontinuous growth process in crustaceans provides an opportunity to study a dynamic form of growth which involves all facets of the animals metabolism. During the molt cycle, and particularly after ecdysis, extensive physiological and biochemical changes take place that affect metal metabolism (i.e. in the hemolymph and digestive gland), and therefore, involves metallothionein [MT] and constitutive metal metabolism. A comparative study of the metal turnover and changes in MT in the blue crab, _Callinectes sapidus_ and American lobster, _Homarus americanus_, during the molt cycle were conducted to determine if similar changes in copper and zinc tissue concentrations occur in both species.

Blue crabs that were examined for changes in metal metabolism during the molt cycle were obtained from commercial crab shedding operations in North Carolina. Both live lobsters and lobster tissues were obtained from the NMFS, Milford Laboratory, Milford, Connecticut. For both species the stage of molt was determined, and hemolymph and digestive gland samples were removed for the analysis of total metal content and cytosolic metal distribution (i.e. in the digestive gland).

Copper and zinc concentrations were determined in both the digestive gland and hemolymph of both species. The metals were wet ashed with concentrated HNO_3 and the metal content measured using flame atomic absorption spectrophotometry.

In both the blue crab and lobster it has been possible to demonstrate significant changes in the total copper and zinc in the hemolymph and digestive gland as a function of the molt cycle. In blue crabs the hemocyanin and copper concentrations in the hemolymph decrease by 60% at ecdysis and copper and zinc also decrease a similar amount in the digestive gland. The changes in lobster are not as large and the timing is slightly different. Through the greater availability of blue crabs we were able to demonstrate short-term change in the cytosolic distribution of copper and zinc in the digestive gland immediately after ecdysis.

Within 90 minutes after ecdysis there were significant changes in the metals bound to MT that were correlated positively with the degradation of hemocyanin. One of the major differences between blue crabs and lobsters from Long Island Sound is the high concentrations of copper in the digestive glands of lobsters (i.e. blue crab, 23 mg/kg and lobster, >500 mg/kg). The lack of large changes in copper among the lobsters that were examined is probably related to the high copper concentrations which makes the detection of subtle changes difficult. A descriptive model has been developed for crustaceans that describes changes in copper and zinc bound to MT and the involvement of MT in the degradation and synthesis of hemocyanin throughout the molting process.

We also have shown that copper/zinc-MT and cadmium-MT are lost from the blue crab digestive gland immediately after molting, but in the lobster little if any of the cadmium is lost from the digestive gland during ecdysis. This difference in cadmium mobilization is underscored by large differences in the binding of cadmium to MT. In lobsters (i.e. Milford, CN lobsters), there is a threshold that must be achieved before cadmium is bound to MT. This threshold cadmium concentration in the digestive gland is 80 to 100 mg/kg, but no such threshold has been demonstrated for the blue crab.

HEMOCYANIN CONCENTRATIONS IN THE HEMOLYMPH OF BLUE CRABS, *Callinectes sapidus*, FROM EASTERN N.C. AND LOBSTERS, *Homarus americanus*, FROM MASSACHUSETTS

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The importance of hemocyanin to health, survival and normal physiological function of blue crabs, lobsters, and all other crustaceans, can not be understated since it serves as the oxygen carrying protein in the hemolymph. The structure and function of crustacean hemocyanins have been studied extensively, and have been shown to be large copper containing proteins composed of a minimum of six subunits each of 75,000 dalton molecular weight. The number of hexamers and their arrangement is species specific.

Extrinsic factors, salinity and oxygen concentration in the environment also can have an influence on the concentrations of hemocyanin in the hemolymph of crabs and lobsters. Decreased salinity and chronic hypoxia or lack of oxygen have been shown to cause an increase in hemocyanin in blue crabs and in lobsters. The information presented here suggests that hemocyanin concentrations also may be affected by environmental factors other than salinity and hypoxia. Evidence that water quality is a forcing function in the synthesis and turnover of hemocyanin in the blue crab and lobster while circumstantial also is quite compelling.

Hemolymph samples were collected from North Carolina blue crabs by the North Carolina Division of Marine Fisheries and from Massachusetts lobsters by the Massachusetts Division of Fisheries for the measurement of hemocyanin concentrations and the determination of its relation to water quality and animal health.

Hemolymph was obtained by either severing the paddle appendage or a walking leg between the joints with a sharp pair of scissors. The samples were collected in plastic vials, placed on ice and allowed to clot. The samples were either sent directly to our laboratory on ice or frozen and shipped by air. The clotted material was broken up and then centrifuged at 20,000 x G for 30 minutes. The resulting serum was then decanted and kept at <4°C until measurements of hemocyanin were made.

The hemocyanin measurements are made spectrophotometrically.

The hemolymph serum samples are diluted with buffer and readings taken at 280 and 335 nm. These readings are compared to standards and the concentration of hemocyanin is calculated.

Blue crabs from the southwestern portion of Pamlico Sound, NC had lower concentrations of hemocyanin in their hemolymph during the summer and early fall of 1989 than did crabs from the two reference areas, Core or Bogue Sound. The lower concentrations of hemocyanin (i.e. reference sites, 43 mg/ml and southwestern Pamlico Sound, 15-25 mg/ml) could not be correlated with the apparent health or sex of the crabs or with salinity. There were, however, statistically significant temporal and spacial differences in hemocyanin concentrations among the crabs that were examined. While low oxygen concentrations did occur in the tributaries of the Sound, they were sporadic and probably were not the only cause of the reduced hemocyanin concentrations.

Lobsters from the coastal waters of Massachusetts, collected in the vicinity of industrialized areas, Boston and New Bedford, had reduced concentrations of hemocyanin relative to the reference areas, Cape Ann and Cape Cod Bay (i.e. 60-70 mg/ml versus 90-100 mg/ml). Among the lobsters that were sampled, however, there was a sex difference that was not consistent. The causes of these differences are unclear which makes concrete correlations between water quality and hemocyanin concentrations more difficult. The general relationship between water quality and hemocyanin concentration that was observed with blue crabs seems to be present among the lobsters that were sampled, but it is not as obvious.

From these observations, we can not determine whether the lowered hemocyanin concentrations were caused by natural phenomena, parasitic disease, or toxic contaminants. It is apparent, however, that the correlations between water quality and hemocyanin concentrations appear to be strong. This correlation is strongest with the blue crab, because we have made measurements in other areas, such as Tampa Bay and the Houston Ship Channel, and the relation between water quality/industrialization and hemocyanin concentration holds. Form our measurements, therefore, it appears that hemocyanin concentration in marine crustaceans may be a useful surrogate indicator of environmental quality, even though the exact mechanisms controlling the system are not well understood.

THE RELATIONSHIP OF SEDIMENT CONTAMINATION TO THE OCCURRENCE OF PRENEOPLASTIC AND NEOPLASTIC LIVER LESIONS IN ENGLISH SOLE (PAROPHRYS VETULUS) FROM VANCOUVER HARBOUR.

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Three years of study have indicated the presence of preneoplastic and neoplastic liver lesions in English sole (*Parophrys vetulus*) from Vancouver Harbour. Compared to the absence of such lesions in English sole in Loughborough Inlet, a relatively undeveloped fjord along the B.C. coast, up to 75% of the fish in Vancouver Harbour were affected by these liver lesions. Highest prevelances were observed in Port Moody Arm, an area of the harbour which has the least tidal exchange and a history of oil refinery among other industrial and urban discharges. Concentrations of selected sediment contaminants, measured in the vicinity of the fish capture sites, were compared to the frequency of liver lesions in the sole. These included polycyclic aromatic hydrocarbons (PAH) which have been linked to fish liver lesions. Since commercial and sport fishing take place in Vancouver Harbour, the prevelance of preneoplasms and neoplasms in the fish raises questions concerning the environmental impacts and potential human health risks.

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Proceedings of the Seventeenth Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, B.C. Vol. 2

Comptes rendus du dix-septième colloque annuel sur la toxicologie aquatique : 5-7 novembre 1990, Vancouver, (C.-B.) vol. 2

Editors/Éditeurs

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Canadian Technical Report of Fisheries and Aquatic Sciences No. 1774 (Vol. 2)

Rapport technique canadien des sciences halieutiques et aquatiques n° 1774 (vol. 2)

Fisheries and Oceans
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Canadian Technical Report of Fisheries and Aquatic Sciences

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Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du ministère des Pêches et des Océans, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications complètes. Le titre exact paraît au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la revue *Résumés des sciences aquatiques et halieutiques*, et ils sont classés dans l'index annual des publications scientifiques et techniques du Ministère.

Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

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PREFACE/PREFACE

The 17th Annual Aquatic Toxicity Workshop was held at the Hotel Vancouver in Vancouver, B.C. on November 5-7, 1990. The theme of the 1990 Workshop was "Threshold Biological Response: Predicting and Determining Ecological Relevance".

The Seventeenth Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the proceedings are published with the support of the Department of Fisheries and Oceans.

The Workshop included 6 plenary presentations, 116 platform presentations, 27 workshop presentations, 25 papers in poster sessions, and several panel discussions. Eight different workshops were held with summaries presented at a final plenary session. Total attendance was 439.

Le 17e colloque annuel sur la toxicologie aquatique a eu lieu les 5, 6 et 7 novembre 1990 à l'Hôtel Vancouver de Vancouver (C.-B.). Le thème choisi pour le colloque de 1990 était : "Le seuil de réponse biologique : son importance pour l'environnement."

Le 17e colloque annuel sur la toxicologie aquatique a permis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces colloques annuels organisés par un Comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec l'aide du ministère des Pêches et Océans.

Le colloques a donné lieu à 6 communications lors de séances plénières, 116 exposés d'invités d'honneur, 27 exposés en ateliers, 25 communications par affichage et plusieurs panels-discussions. Les résumés des huit ateliers ont été présentés à la séance plénière finale. 439 personnes ont assisté au colloque.

EDITORS' COMMENTS/ REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and a list of participants are also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur; ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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The organizing committee thanks the following and acknowledges the sponsors for their financial and/or other support.

Many persons worked to make the workshop a success. We extend our thanks to all of them, in particular to the volunteers, authors, session chairpersons and the participants for their contributions. We also extend our gratitude to the staff of E.V.S. Consultants and of the Hotel Vancouver, Vancouver, B.C., for their cooperation and efforts for the workshop.

Le comité d'organisation tient à remercier les commanditaires de leur soutien financier ou autre.

Nos remerciements vont aussi à toutes les personnes qui ont contribué au succès de colloque, en particulier aux travailleurs bénévoles, aux auteurs, aux présidents des diverses séances et aux conférenciers qui ont présenté leurs travaux. Enfin, nous voulons exprimer toute notre gratitude au personnel de E.V.S. Consultants et de l'Hôtel Vancouver, de Vancouver (C.-B.), pour leur collaboration et leur excellent travail lors du colloque.

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PLATFORM SESSION

Chemicals, Scientists & Managers

Chair: J. Young

CHEMOPHOBIA

or

THE DEATH OF SCIENCE

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by

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Chemophobia and risk perception

The environment has become a major, worldwide topic for discussion as evidenced by conferences like Globe 90 in Vancouver and lengthy articles in publications such as The Economist[1]. Concurrent with that discussion has been an increased public concern about environmentally related risks in general, and chemical risks in particular[2]. Unfortunately, the public often has an irrational fear of chemicals in the environment, a perception considerably different from the risks assigned by experts[2,3,4]. This fear, which Huntzinger calls "chemophobia", was the subject of a symposium in Bavaria in 1985 with chlorinated dioxins as the example[5].

It is difficult to reassure the public that many of these perceived risks are relatively insignificant (e.g., pesticide residues in food), and equally difficult to convince people to accept some risks as serious (e.g., smoking, radon gas). There are several reasons for these differences in the outcomes of the informal risk assessments done by the public and those performed by experts.

Risk versus hazard

The first reason is that risk means different things to the public and to professionals involved in risk management. The public and media confuse risk with hazard, and often incorrectly assume that where there is a hazard, there is a significant risk. A hazard is defined as any possible adverse event and risk as the likelihood of the event occurring. Toxicological risk assessment is the process of using toxicological, epidemiological, environmental fate and exposure data to calculate risks associated with chemical hazards.

Public ignorance

A second and major factor for the difference is that lay people are amazingly unfamiliar with technical topics and indeed, shockingly ignorant of basic science. A recent article in the Globe and Mail, some 400 years after Copernicus, reported that 20% of Canadians believe the sun orbits the earth and 49% do not know how long it takes the earth to circle the sun[6]. Human and environmental risk assessments are a complex process, and the general public has little understanding of that process.

There is a large reluctance by the public and activist groups to accept that familiar and traditional use of chemicals poses a very high level of risk. Ames (1987) and others[7] have compared the risks from the ingestion of synthetic pollutants to the risks of consuming natural and traditional carcinogens. They conclude that more than 99.99% of the pesticides ingested are produced naturally by the plants we eat, and over 50% of all chemicals tested - natural or man-made - are carcinogenic at high doses. "Organic" farmers selecting naturally resistant cultivars, are thus selecting the ones with the highest levels of natural pesticides; many of these are potent carcinogens.

The evidence shows that cancer rates are not increasing; age-adjusted cancer rates are either stable or declining, except for melanomas due to excessive suntanning and smoking related cancers[4]. Despite the scientific literature, government reports and the urging of public health groups, the media and the public pay little attention to the risks of an improper diet, the abuse of cigarets and alcohol, and suntanning.

Table 1. Public v/s U.S. EPA Risk Concerns

EPA's List of Concerns
(not ordered by EPA)

Ecological Risks

- global climate change
- ozone depletion
- habitat alteration
- species extinction and loss of biodiversity

Health Risks

- "criteria" air pollutants (e.g., smog)
- "toxic" air pollutants (e.g., benzene)
- radon gas
- indoor air pollution
- drinking water contamination
- occupational chemical exposures
- pesticide application
- ozone depletion

Public Ranking of Concerns

1. active hazardous wastes sites
2. abandoned hazardous wastes sites
3. water pollution from chemicals
4. occupational exposures to toxic chemicals
5. oil spills
6. ozone depletion
7. nuclear power plant accidents
8. pollutants from industrial accidents
9. radioactive wastes
10. industrial air pollution
11. leaking underground storage tanks
12. coastal water contamination
13. solid waste
14. pesticide exposures for farm workers
15. water pollution from agricultural runoff
16. water pollution by sewage plants
17. vehicle air pollution
18. pesticide residues in food
19. greenhouse effect
20. drinking water contamination
21. destruction of wetlands
22. acid rain
23. water pollution from urban runoff
24. nonhazardous waste sites
25. biotechnology
26. indoor air pollution
27. radiation from X-rays
28. indoor radon gas exposures
29. radiation from microwave ovens

NOTES:
- underlined items also appear on the EPA list
- EPA list as identified by the EPA Scientific Advisory Board
- public list from April 1990 poll

adapted from Roberts (1990) (reference 2.)

Risk and outrage

While risk assessment is supposedly divorced from emotion, people base their risk perceptions on subjective criteria in addition to their own estimates of mortality[7]. The public will minimize risks when they are perceived to be voluntary, controllable by the individual, familiar, easily reducible, decreasing, and non-catastrophic. Conversely, when they are perceived to be dreadful, fatal, unfamiliar, uncontrollable by the individual, unfair, involuntary and potentially catastrophic, risks are usually of great concern to the public[7,8]. These psychological and political aspects have been termed risk "outrage" by Sandman (1990) who argues that public concerns are often more a function of outrage than actual risk. People consistently underestimate the risk of low-outrage hazards and overestimate the risk of high-outrage hazards. "Experts" outside of their field of expertise make similar errors[8].

Chemical risks fall into the "outrageous" category for several reasons. Many people view "chemicals" as exclusively man-made, the result of technology. The public views technology as new, unfamiliar, artificial and potentially upsetting to a presumed "natural balance.". They are not aware that the overwhelming majority of chemicals they come into contact with are produced by nature and the human body is unable to differentiate between a natural and a man-made chemical. In addition, exposures are often involuntary, and exposures to small quantities of some chemicals, both synthetic and man-made, can result in dreadful outcomes (e.g., death, cancer, birth defects). By association and ignorance, these dreadful outcomes are attributed especially to man-made chemicals.

There are three different types of chemical exposures and different risks are associated with each: (1) true disasters such as Bhopal; (2) accidental spills and occupational exposures which result in acute exposures; and (3) exposure to trace contaminants such as pesticide residues and chlorinated dioxins in food, air and water[7]. The public and the media fail to distinguish amongst these exposures and consequently attach to each the worst features of all three.

The media

The public relies on the media for most of their information on environmental issues[9]; however, the media are also frequently naive with respect to toxicological issues and ignorant of technical topics[7,8] . The media has also been accused of taking an advocacy rather than objective role[10] and paying more attention to the emotional side of an issue rather than the technical aspects[8]. The distinct impression often left is that the media are often more interested in ratings or circulation than in conveying balanced facts. By focussing on these emotional aspects, the media help to build and focus outrage. Given their lack of technical understanding, there is a strong likelihood this outrage will involve the wrong hazard.

Industry contributions

The public's perception of the risk associated with chemicals has been heightened by industry. Incidents like Bhopal and Soveso have convinced the public that industry is concerned only with the pursuit of profits. Industry association with a handful of fraudulent studies has served to impugn the credibility of the great majority of responsible and honest researchers.

On a more subtle scale, industry representatives have failed to recognize that while the public is largely ignorant of technical matters, people are capable of understanding complex issues if they are explained. Outrage has thus been increased by the failure to provide

Table 2. Factors Affecting Risk Perception

HIGH PERCEPTION FACTORS	LOW PERCEPTION FACTORS
- affects me	- doesn't affect me
- not observable	- observable
- unknown to those exposed	- known to those exposed
- new risk	- old risk
- risks unknown to science	- risks known to science
- uncontrollable	- controllable
- involuntary	- voluntary
- dreadful	- not dreadful
- effects delayed	- immediate effect
- global catastrophe	- not globally catastrophic
- fatal	- not fatal
- not easily reduced	- easily reduced
- risk increasing	- risk decreasing
- high risk to future generations	- low risk to future generations
- not equitable	- equitable
- widespread	- individual

adapted from Scheuplein (1989) and Sandman (1990) (references 7. & 8.)

explanations, the failure to recognize that the public's definition of risk includes outrage, and the failure to address outrage in addition to the experts' traditional risk assessment[8].

Government agencies

Government agencies build outrage unintentionally through the use of inappropriate or worst case scenarios. Virtually every regulatory risk assessment is qualified by some statement that the risk estimate represents the upper bound of the likely risk, the estimate probably overestimates the risk, and the true risk may be as low as zero[11]; however, the upper bound estimate is often accepted as the real risk. Both the U.S. Environmental Protection Agency (EPA) and the National Academy of Science have published worst case scenarios for pesticide residues in food which give the strong impression that thousands of deaths per year can be expected from consuming foods with currently allowable levels of pesticide residues[7].

Paustenbach (1990) observes that "Often, regulatory agencies and the press have claimed or implied that the results of low-dose models actually predict the increased cancer risk for exposed individuals." Dr. Young of the US Food and Drug Agency clarified the issue[12] :

> "The risk level of one in a million is often misunderstood by the public and the media. It is not an actual risk - i.e., we do not expect one out of every million people to get cancer if they drink decaffeinated coffee. Rather, it is a mathematical risk based on scientific assumptions used in risk assessment. ... When FDA uses the risk level of one in a million, it is confident that the risk to humans is virtually nonexistent."

Regulatory agencies have also been criticized for using the maximum tolerated dose to directly estimate human risks at low doses[11,13]. The extensive data base compiled by Ames, et al. (1987) shows that more than half of all chemicals tested, synthetic and natural, were carcinogens in both rats and mice at the maximum tolerated dose commonly used in cancer bioassays. Both Ames, et al. (1987) and Scheuplein show that at current exposure levels, cancer risks from many natural foods are much higher than the risks associated with synthetic chemicals.

In their epidemiological analysis of U.S. cancer mortality records which are summarized by others7[14], Doll and Peto (1981) attributed about 2 percent of total cancer mortality to pollution and about 3 percent to geophysical factors (sunlight and other natural radiation). Of the rest, 35 % was assigned to diet and 30 % to tobacco consumption. Gough (1989) reports these results, based on cancer mortalities, to be comparable to estimates of mortality derived from a risk assessment report published by the U.S. EPA.

Environmental activist groups

Activist groups build outrage intentionally; low outrage hazards don't do much for memberships or financial contributions[7,8]. Unfortunately, both the public and the press rely on and trust activist groups for environmental information[9,10]. Huntzinger is quoted as saying the problem of chemophobia is due to "instant self-appointed experts[5]", thus activist groups arguably are the largest current reason the public incorrectly judges the risks of chemicals.

When medical authorities Dr. Shaun Peck and Dr. John Blatherwick attempted to put chemical risks in perspective[16], Greenpeace was highly critical. Renata Kroesa, a Greenpeace official, was quoted in Western Report magazine (1989) as saying

Table 3. Causes of Cancer Mortalities

	Percent of all cancer deaths		
	Doll & Peto		
Exposure category	best estimate	(range)	U.S. EPA
diet	35	(10-70)	
tobacco	30	(25-40)	
sexual behavior	7	(1-13)	
occupation	4	(2-8)	<1-4
geophysical factors	3	(2-4)	3-6
alcohol	3	(2-4)	
pollution	**2**	**(<1-5)**	**1-3**
medicine/medical procedures	1	(0.5-3)	
food additives	<1	(-5-2)	
industrial products	**<1**	**(<1-2)**	**<1**
infection	10?	(1-?)	
unknown	?	(?)	

adapted from Scheuplein (1989) and Gough (1989) (references 7. & 14.)

"It is obvious that Dr. Peck has fallen victim to an industry-backed campaign to discredit environmentalists. It's really just one big public relations campaign[17]."

One of the best specific examples of outrage building and exaggeration of risk is chlorinated dioxins[5]; although, there are other examples such as the greenhouse effect and nuclear power. There is an increasing agreement amongst the scientific community that the health risk of chlorinated dioxins has been grossly overestimated. Ames (1989), Leung, et al. (1988) and Tschirley (1986) discuss the carcinogenicity of dioxins in some detail. Comparing the carcinogenic potential of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to that of ethyl alcohol, a known teratogen and carcinogen, indicates that the risk of consuming the EPA reference dose of 6 femtograms/kg/day of TCDD is equivalent to the risk of consuming 1 beer every 8000 years. Further, while the binding of TCDD to receptors in mammalian cells has been cited as a reason for concern, a variety of other substances including polycyclic hydrocarbons in cooked meat and indole carbinol in broccoli and cabbage also bind to the same site.

Chlorinated dioxins are not genotoxic, thus there is every reason to believe that exposures follow a dose-response relationship. Some 500 years ago Paracelsus (1493 - 1541) noted: "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy[21]." In contrast, Mark Floegel of Greenpeace is quoted in The Washington Post (1990) as saying:"The only acceptable standard for dioxin is zero[22]."

Advocacy groups have also not hesitated to prevaricate on the dioxin issue. B.C. Cancer Control Agency data were used by the Western Canada Wilderness Committee to link female lung cancer deaths with pulp mills in the Howe Sound area. Paul George, a Director of the group is quoted in Vancouver Magazine (1990):"...bogus...it was totally prefabricated by us to try to tell people, to scare people that air pollution stinks....that dioxin-filled little particles that collect on your cars and rust them out...hurts your lungs...[23]."

A modest proposal

At the 1985 Bavarian symposium on chemophobia, Simmler is quoted as saying: "Chemophobia is justified by industry--formalized by government--nourished by the media--tolerated, at least, by politicians--kindled by whoever expects personal or collective gains of sorts[5]." Potential solutions to the problem of chemophobia have been discussed by Weinberg (1985) and Sandman (1990). In addition to tentative solutions, Weinberg (1985) offers detailed review of the role of science and uncertainty in environmental issues and regulation. Sandman (1990) addresses the need for and methods of risk communication.

There are essentially four corrective elements for chemophobia. First, the scientific community must be instrumental in helping decide what constitutes proof in complex environmental issues and what constitutes adequate scientific quality assurance in these issues.

Second, scientists, regulatory officials and the media must decide how to best educate the public about risks, including what constitutes the serious risks. They must also decide how to inform the public that neither science or engineering can provide 100 % certainty in some issues.

Third, the scientific community and the media must place more emphasis on those risks which are serious. By emphasizing those risks, the public will be willing to help reduce them and politicians willing to allocate the necessary resources.

Fourth, everyone, activists groups and regulatory agencies included, must stop emphasizing the insignificant risks, and the press must stop reporting insignificant risks as serious.

As Peck and Blatherwick (1990) state: "Involuntary risks are an unavoidable aspect of human society; minimizing those risks is the hallmark of a sane and humane society. Resources for this effort are limited. Surely it is necessary to ensure that these limited resources are expended where the greatest benefits to public health and the environment can be achieved."

Table 4. Relative Risks From Potential Carcinogenic Hazards

Source	Contaminant	Relative risk
PCBs (daily dietary intake)	Polychlorinated biphenyls	0.0002
EDB (daily dietary intake)	Ethylene dibromide	0.0004
Tap water (1 L)	Chloroform	0.001
Bacon (100 g cooked)	Dimethylnitrosamine Diethylnitrosamine	0.003 0.006
Swimming pool (1 hr. for child)	Chloroform	0.008
Peanut butter (1 sandwich)	Aflatoxin	0.03
Diet cola (12 oz.)	Saccharin	0.06
Basil (1g dried leaf)	Estragole	0.1
Phenacitin pill	Phenacitin	0.3
Home air (14 hr/day)	Formaldehyde benzene	0.6 0.004
Beer (12 oz.)	Ethyl alcohol	2.8
Wine (250 ml.)	Alcohol	4.7
Formaldehyde (occupational exposure)	Formaldehyde	5.8
EDB (heavy occupational exposure)	Ethylene dibromide	140

NOTES
- EDB = ethylene dibromide
- PCB = polychlorinated biphenyls

adapted from Ames, et al. (1987) (reference 13.)

Table 5. Naturally Occurring Carcinogens in Foods

Food	Carcinogen	Concentration
Mushrooms	Hydrazines	400 - 500 ppm
Parsnips & celery	Psoralens	40 ppm 0.8 - 25 ppm
Cereals, corn, seeds, nuts	Aflatoxin	1 - 400 ppb
Herbal teas	Pyrrolizidine alkaloids	0.01 - 0.6 %
Spinach, beets lettuce, radishes	Nitrates/nitrosamines	0.1 - 0.2 %
Fish & shellfish (via phytoplankton)	Polycyclic hydrocarbons	2 ppm
Orange juice	d-limonene	31 ppm
Nutmeg	Safrole	3000 ppm
Basil	Estragole	3800 ppm
Honey	benzyl acetate	15 ppm
Brown mustard	Sinigrin	16000 - 72000 ppm
Fruits	Caffeic acid	50 -200 ppm

adapted from Scheuplein (1989) and Ames (1990) (references 7. & 25.)

Table 6. Risk Estimates of Various Foods Categories Containing Carcinogens

Food Category	Examples	% of Risk
Traditional food	Grains, fruits, meat, etc.	41.32 - 98.82
Spices & flavors	Mustard, pepper, vanilla,etc.	0.98 - 4.13
Indirects	Packaging migrants, surface residues, etc.	0.20 - 8.26
Pesticides & contaminants	Insecticides, PCBs, dioxins, etc.	¯0.01 - 0.41
Animal drug residues	Antibiotics, growth promoters, etc.	¯0.01 - 0.41
Food preparation (charred protein only)	Broiling, baking, etc.	¯0.01 - 41.32
Mycotoxins	Aflatoxins, ochratoxin A, etc.	¯0.0001 - 4.13

NOTES - range of cancer risk depends upon different assumptions regarding carcinogenic potencies

adapted from Scheuplein (reference 7.)

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ENHANCEMENTS TO NOAA'S STATUS AND TRENDS PROGRAM (NSTP). TK Collier, JE Stein, SD Connor, LL Johnson, E Casillas, and U Varanasi. Environmental Conservation Division, NWFC, NMFS, NOAA. 2725 Montlake Blvd. E. Seattle, WA, USA (206)442-1432.

The NSTP has recently been expanded to allow better determination of the relationships between toxic chemicals and associated biological effects in coastal ecosystems. One area of enhancement has been the incorporation of more sensitive biological effects, such as xenobiotic-inducible enzyme activity in benthic fish species. The results from two years of measurement of isoenzymes of cytochrome P-450 (P450) or P450-dependent mixed function oxidase (MFO) activities show that this enzyme system is generally responsive to contaminant exposure in several species from both the East and West Coasts. However, additional studies (e.g. dose-response) are planned in order to better interpret the results. Another area of enhancement to the NSTP is the recent evaluation of reproductive dysfunction as a possible serious biological effect associated with exposure of benthic fish to toxic contaminants. In a series of field studies, we have shown that impaired ovarian maturation and failure to spawn can result from contaminant exposure, and we have found that levels of plasma estradiol and hepatic MFO activities are useful predictors of these effects.

INTEGRATION OF ECOTOXICOLOGICAL DATA FOR INDUSTRIAL EFFLUENT EVALUATIONS. R. van Collie, N. Bermingham, C. Blaise, G. Costán, and R. Vezeau. Environment Canada, St. Lawrence Centre, Ecotoxicology. 1001 Pierre Dupuy, Longueuil, Québec J4K 1A1 (514) 651-6860.

Depending on the specific purpose, a relatively exhaustive cause and effect study or a quick managerial look for selected potential effects is suggested. For the first concern, we have developed a multidisciplinary, tiered and integrated hazard assessment scheme combining physico-chemical and bacteriological analyses as well as biotests representative of lethal, sublethal and chronic effects. This scheme results in identifying local, zonal and regional potential scope of impact. Application of this approach necessitating an average of $6,000 per study and one full month to conduct all the tests is presented with the case study of a petrochemical effluent. For the second and evidently less demanding concern, we suggest our potential ecotoxicological effects ready reckoner scale. This "PEER" scale combines both toxic and genotoxic effects. Values integrate effect end points, intensity of response (slope), persistence and flow for a given industrial effluent. Application of this "PEER" scale is presented for St. Lawrence River action plan concerns. Cost and time for procurement of information is reduced to $2,000 and 1 week. These two distinct applications show how ecotoxicology can and must adapt to the varied needs of environmental managers.

CHEMICAL ANALYSES: HOW EXACT ARE THEY?

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The demand for high-quality analytical laboratory capabilities has grown significantly as environmental regulatory agencies have built the inventory of "toxics" lists and continue to enhance the comprehensiveness of environmental legislation. There is a dramatic trend toward multicomponent determinations at very low concentrations in complex sample matrices. These requirements place an increasing burden on the laboratory and its ability to produce quality results.

Analytical environmental chemists must clearly understand and provide information concerning the limitations of their data. Regulatory personnel in particular must be acutely aware of the limitations of environmental testing. It is important that this awareness and understanding be passed on to the public and the press to prevent common misrepresentations.

The U.S. Food and Drug administration evaluates analytical methods based on three characteristics (Horwitz, 1982): reliability; applicability to a wide variety of sample types; and practically with respect to cost, time, and training constraints.

Reliability is the overriding consideration and is determined by establishing the following characteristics of a method.

- Reproducibility is the between-laboratory precision of an analysis determined by interlaboratory, or "round robin" studies. The real aim of interlaboratory tests is to determine the allowance for variability, expressed as a coefficient of variation, that must be made among laboratories to make the values interchangeable.

- Repeatability is the within-laboratory precision and defines the ability of a laboratory to check itself. It can be expressed as a coefficient of variation and should be better than the reproductivity. In most studies, within-laboratory variability is about two-thirds of the interlaboratory. Methods for which the reproducibility is substantially higher than within-lab variability would be considered very personnel dependent and not good for some purposes.

- Accuracy, or bias, is how close a measured value is from the true value. Some analyses have a well-defined, known error called a systematic error.
- Specificity is the ability of a method to measure an analyte, without interference from other chemicals.
- Sensitivity or the analytical detection limit is defined as the smallest amount of an analyte that can be measured with a stated confidence.

Chemical analyses are required for three major purposes (Maynard, 1989):

- To survey in order to determine the extent or nature of contamination. A high degree of accuracy and precision is not always necessary, as the object is to determine if analytes are present and whether their levels are high or low.
- To monitor. Monitoring requires numerous determinations with practical methods that can provide sufficient information in the time allotted. The methods should be rapid and precise enough to determine if significant concentrations are present on a significant number of occasions.
- To comply with legal requirements. Compliance monitoring requires a high degree of accuracy and precision. It is necessary to select a few parameters that are analyzed by well-defined, validated methods.

As an example of the above, consider the requirement of a regulatory agency that must set a comprehensive discharge permit for a particular industry with an unknown effluent quality. The first step would be to establish what is present and absent in the wastewater through a comprehensive characterization of the effluent. Multicomponent tests would be best for this purpose, for they involve measuring numerous parameters at one time (i.e., generalized extraction followed by gas chromatography/mass spectrometry for organics; inductively coupled argon plasma for metals). The second step would be to monitor at a defined frequency using more specific analysis methods to establish overall variability of discharge and establish possible relationships among parameters. This would include measurements of physical parameters that define the condition of the water with respect to its suspended matter, conductivity, color, oil and grease, and oxygen demand, and analyses for specific parameters such as nutrients, anions, metals, and individual organics. Finally, a short list of parameters would be established for compliance monitoring using well-defined specific methods.

The methods selected must be continuously defined through a comprehensive quality assurance program. A well-managed laboratory will devote at least 15-20% of its effort to quality assurance, the components of which are discussed by Maynard (1989).

The interlaboratory (between laboratory) variability associated with a number of environmental tests has been reviewed by Maynard (1989). It was demonstrated that many tests such as metals, key conventionals, and some organics, can be accurately and precisely determined while others such as oil & grease, total phenols and certain individual organics can be associated with a high analytical variability.

The variability introduced by sampling and sample preparation can be very significant. This was recently discussed by Deverall et al (1989) in a study to characterize sampling and sample preparation procedures prior to analysis of harbour sediments. These sediments were collected from five marine harbours on the West Coast of British Columbia. The sites were chosen to reflect a wide variety of sample types from clean muds to highly contaminated materials. The study concluded that although good laboratory procedures are imperative, field sampling and sample preparation methods can have a greater influence on the data generated.

PLANNING IS ESSENTIAL.

A well-run program will generate meaningful data required for research, survey and regulatory purposes. Project managers and field staff must communicate with laboratory personnel to discuss the important components of any analytical program:

- sample collection and storage procedures
- decisions regarding representativeness of the samples. In some waste evaluation studies, nonhomogeneous samples are collected.
- parameter list, which should be narrowed down as much as possible.
- detection limit requirements
- scheduling, because quality analytical work takes time to complete

- report requirements

Careful attention to the limits of the procedures used, quality control performed, and cooperation between all parties involved will ensure the data provides meaningful information.

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DURATION OF EXPOSURE, ITS ROLE IN ASSESSING TOXICITY OF EFFLUENTS, AND THE NEED TO RECOGNIZE THE BENEFITS INHERENT IN WELL-SITED AND WELL-DESIGNED OUTFALLS

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ABSTRACT

The Environmental Protection Agency (EPA) is pressuring the states to establish requirements in permits for measuring and limiting toxicity. Washington State is developing biomonitoring requirements without well validated protocols, without concern for economic impacts, and without recognizing that some sensitive species are protected from acute exposures by physical parameters of effluent dispersion related to outfall design and location.

EPA offers some relevant guidance through its Technical Support Document (TSD) for Water Quality-Based Toxics Control (EPA 1985). The TSD is undergoing a sizeable expansion and a draft TSD was published in April 1990 (EPA 1990). Both versions offer ways to define where acute toxicity criteria (either for specific toxicants or for effluent toxicity) must be met. Washington is incorporating the TSD approach into their own mixing zone strategy.

For a well designed outfall with a diffuser that can achieve very rapid dilution, the compliance area for meeting acute criteria may be very close to the discharge. A discharge with diffuser ports of 5 centimeters in diameter would be required to meet acute criteria (both for numeric concentrations and for "toxicity") at a distance of less than three meters.

Given this consideration, acute toxicity measurements using organisms that are not strong swimmers is unrealistic. A drifting organism would have an exposure to high concentrations of effluent for just a matter of seconds and could not conceivably re-enter the plume for sufficient repeat exposures. In such situations only fish species are appropriate for evaluating toxicity, even if other species are more sensitive in laboratory tests. Regulatory programs that ignore this benefit of siting and design will force unnecessary toxicity controls that will have little or no environmental benefit.

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[1] Served on Department of Ecology advisory committees regarding mixing zone regulation and biomonitoring policy.

[2] Served on Department of Ecology advisory committee regarding mixing zone regulation.

MIXING ZONES, REALITY VERSUS PUBLIC PERCEPTIONS

Assessment of toxicity, either by numeric criteria or by means of bioassays, must be related to a mixing zone regulatory framework to be put into a management context. Many in the public who do not understand effluent dilution, and who have been taught that "dilution is not a solution to pollution" oppose any mixing zones. Perhaps in some locations that might be prudent, but certainly not in water bodies where volumes are large, flow is great, and harm is improbable from the particular effluent.

Failure to allow use of mixing zones would result in all present (and future) standards being applied to effluents at the end of pipe. Such a policy could result in greater environmental harm because of focusing heroics on one receiving media (surface water) which could result in greater harm elsewhere (such as from increased energy needs in treatment, increased site impacts from larger treatment facilities, and cross-media impacts in which air, land or groundwater could receive greater loadings). For example, a primary sewage plant may require little energy, or produce its own energy, whereas a secondary treatment sewage plant would require considerable energy and produce twice as much sludge requiring either land disposal or incineration.

The Department of Ecology (DOE) in Washington has been developing a mixing zone regulation (DOE 1990b), and developing a biomonitoring policy (DOE 1990a). These regulatory programs are being developed separately, yet their interaction is important. Advisory committees have assisted the DOE in this process.

DOE's draft mixing zone regulation (DOE 1990b) establishes maximum sizes for mixing zones, insists that mixing zones be as small as possible, and requires that the size of the zone should be reassessed at each discharge permit renewal. However, no guidance exists to permit writers as to how to establish the dimensions of a smaller mixing zone. There is a strong (but erroneous) perception among some groups that Puget Sound is covered with large mixing zones in which all the waters are toxic to all marine life. Under this misperception, reduction of mixing zones is considered as one more regulatory method to improve water quality, presumably by forcing additional treatment measures. Dischargers could be faced with a seemingly endless string of dilution ratio studies under this plan.

One representative on the citizen's advisory committee for the state's biomonitoring policy voiced his opposition to mixing zones by saying these have got to stop, it's about time all the dischargers were made to, "pay the piper". His perception obviously was that mixing zones were areas of great pollution and hideous impacts to aquatic resources. It is a perception that the public can be easily led to believe, but it is a far cry from reality.

WASHINGTON STATE'S DRAFT MIXING ZONE REGULATION

DOE has been considering its mixing zone policy for the last three years, and has been developing specific regulatory language in the last year (DOE 1990b). Presently in draft form, it is scheduled for adoption in summer 1991. Briefly, it details siting considerations for different receiving waters, such as lakes, rivers, estuaries and marine waters, seeks to avoid having mixing zones for discharges located near sensitive areas and limits the amount of the water body width that should be used.

Maximum mixing zone dimensions in rivers and in marine waters would be horizontal distances from the discharge equal to 300 feet (91 m) plus the water depth. In estuaries it would be 200 feet (61 m) plus the water depth. In rivers and estuaries there is a preference that mixing zones not occupy greater than 25% of the cross sectional width of the water body, but exceptions can be made to this. Chronic criteria, both for individual constituents for which numeric criteria existed in state regulation, and for whole effluent toxicity, would apply at the edge of the mixing zone.

Acute criteria would apply within the mixing zone close to the discharge itself, but not at the end-of-pipe. The draft mixing zone regulation follows the guidance in EPA's draft TSD. The specific wording is:

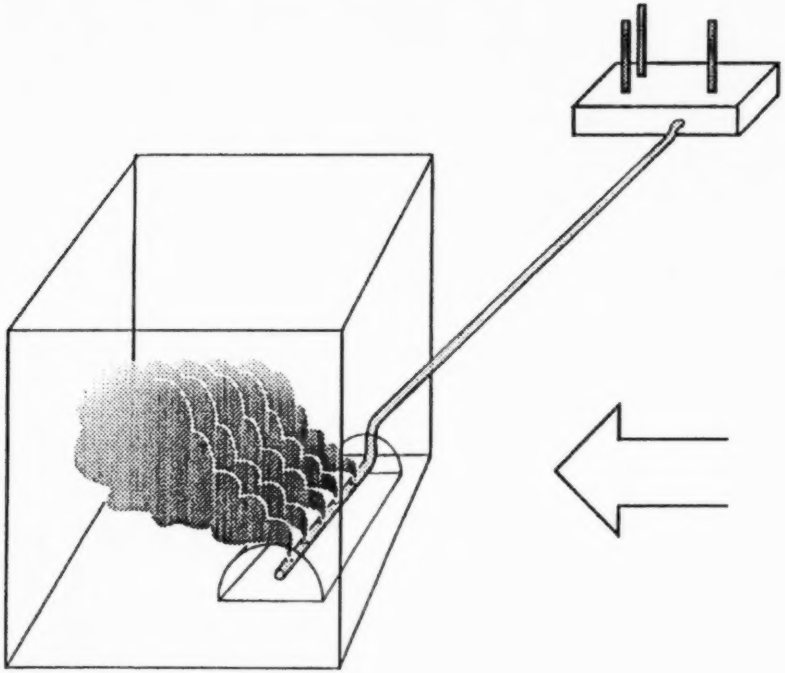
> A zone of acute criteria exceedance is allowed only if it can be demonstrated to the department's satisfaction that the concentration of, and exposure duration and frequency to, the discharge will not create a barrier to the migration or translocation of indigenous organisms to a degree that has the potential to cause damage to the ecosystem. Acute criteria (based on numeric criteria and bioassays approved by the department, as generally guided under WAC 173-201-047 (1) through (4)) shall be met, as near to the point of discharge as practicably attainable. Compliance shall be determined by monitoring data or calibrated models approved by the department and/or by the use of bioassays utilizing representative dilution ratios. In no case shall the zone where acute criteria may be exceeded be greater than the most restrictive of the following:
>
>> (i) Ten percent (10 %) of the distance from the edge of the outfall structure to the furthest horizontal edge of an authorized mixing zone, as applied in any spatial direction.

> (ii) Fifty (50) times the discharge length scale in any spatial direction from each discharge port (the discharge length scale is the square-root of the cross-sectional area of any discharge outlet). In the case of multi-port diffusers, this requirement must be met for each port using the appropriate discharge length scale of that port.
>
> (iii) Five (5) times the local water depth, during critical hydraulic conditions, in any horizontal direction from any discharge outlet. The local water depth is defined as the natural water depth (existing prior to the installation of the discharge outlet) prevailing under critical conditions."

Figure 1 provides an illustration of a hypothetical mixing zone typical of a river discharge where the direction of flow is constant. The length would be 300 feet plus the water depth. The plume itself would occupy only a portion of the volume in the mixing zone, and that can be calculated for different rates of discharge, ambient currents and water column densities. The chronic criteria would have to be met where the plume crossed any of the planes defining a side of the mixing zone. Presently Washington state has surface water numeric criteria for 25 toxicants which in turn are based on EPA criteria (EPA 1986). DOE is currently reviewing its water quality standards and may add to this list in 1991. Eventually the state will implement some method whereby numeric criteria for whole effluent chronic toxicity, based on biomonitoring, must also be met at this location.

Figure 1 also shows a hypothetical example of where the "zone of acute, criteria exceedance" would be. This will include whole effluent acute toxicity when DOE promulgated regulations for numeric standards. Notice, that instead of a box, it is a curved shell surrounding the diffuser at, or closer than 1/10th the distance from the diffuser to the edge of the mixing zone. As can be seen from this figure, a mixing zone is not a large volume of toxic soup as some members of the public may perceive. The volume of water in which the acute criteria could be exceeded is very small. Furthermore, with many effluents, the acute criteria might actually be met even closer to the diffuser. This is the case for many major dischargers now. Describing the zone in which acute criteria may be exceeded does not mean that exceedances occur throughout the zone, or even occur at all. Furthermore, exceedance of an acute criteria at the edge of the zone (or even beyond the edge of the zone) does not mean that organisms in the receiving water are impacted.

Figure 1.
A Hypothetical Mixing Zone



- Chronic Criteria Apply at Edge of Box
- Acute Criteria Apply at Edge of Curved Shell About Diffuser

Figure 2 presents an enlarged view of this hypothetical zone of acute criteria exceedance. Even within this zone, not all the water volume has high effluent concentrations. Discharge ports are separated by design determined distances to prevent the individual plumes from combining before a high degree of mixing has already occurred. The figure is actually a simplification by showing the zone as a section of a cylinder over the outfall. Actually it would be an even smaller volume defined by the intersection of portions of spheres, where each is centered over a diffuser port. Some outfalls have different size diffuser ports along the outfall, and the zone of acute criteria exceedance would then be both convoluted and also distinctly fatter and skinnier in places. You may gather that this could pose some unusual difficulties in monitoring by any means other than with divers.

Condition (ii) described above will determine the distance to the acute zone boundary for almost all marine outfalls with diffusers. According to EPA's TSD (EPA 1985 and 1990), if the limiting condition is condition (ii), it will ensure a dilution factor of 10 or better. That means that the concentration is reduced to 10% effluent (or less) at that point. Actual field observations would probably yield even greater dilution at that point.

Figure 3 presents a two dimensional representation of the zone of acute criteria exceedance, an outfall diffuser and the plume passing through the zone of acute criteria exceedance. A second arc is also shown for purposes of discussion. Table 1 shows just how close to a diffuser this distance is if, as is the case for most outfalls, condition (ii) from EPA's TSD (EPA 1985 and 1990) and the draft state mixing zone regulation (DOE 1990b) determines the zone.

TABLE 1

Radius of zone of acute criteria exceedance for different port sizes

Discharge port size	Radius of zone of acute criteria
5 cm (2.0")	2.2 m (7'3")
10 cm (3.9")	4.4 m (14'6")
15 cm (5.9")	6.6 m (21'9")
20 cm (7.9")	8.8 m (29'0")
25 cm (9.8")	11.0 m (36'3")

(distances determined by 50 x square root of discharge port area)

In looking at Figure 3, it is important to recognize that this is a very small distance from an outfall, and that very

Figure 2.
A Hypothetical Acute Mixing Zone

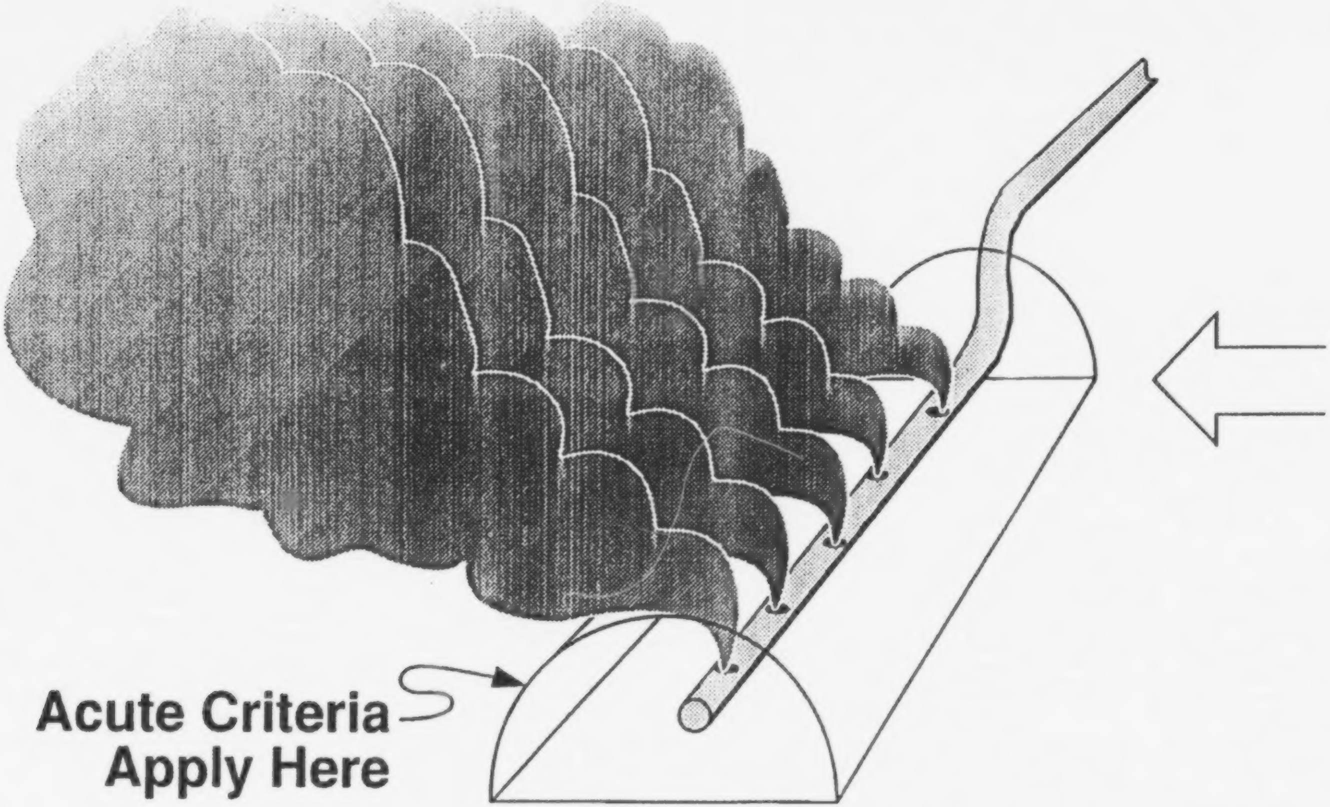
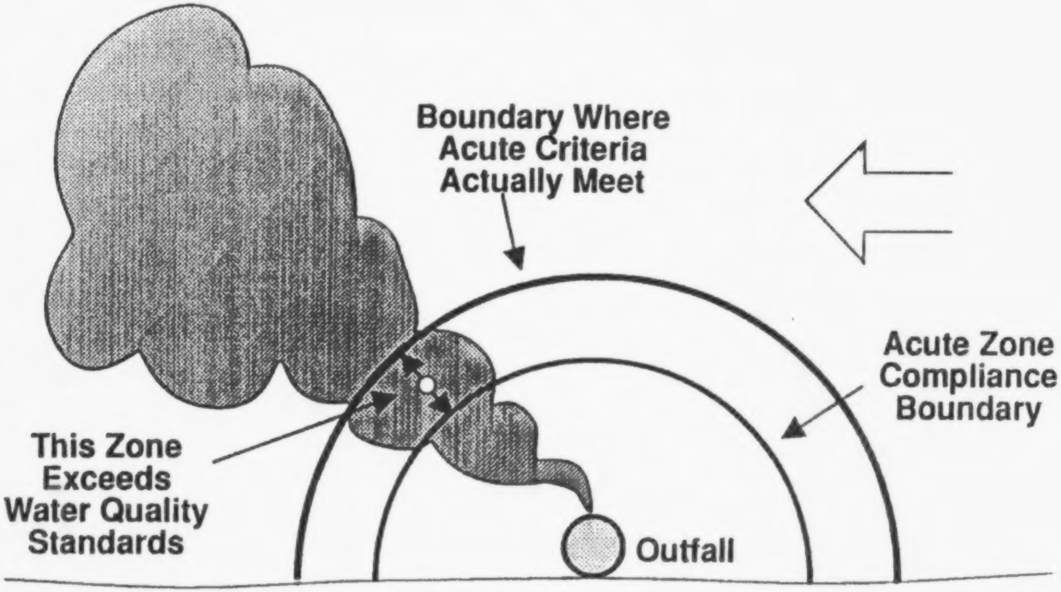

Acute Criteria
Apply Here

Figure 3.

Two Dimensional Cross Section


Boundary Where
Acute Criteria
Actually Meet
Acute Zone
Compliance
Boundary
This Zone
Exceeds
Water Quality
Standards
Outfall

Hypothetical Area of Non-Compliance with Acute Water Quality Standards for Toxicants

Parametrix, Inc.

rapid dilution occurs within this small distance. It is also important to recognize that current velocities within this distance, associated with the turbulent flow induced by jet action and buoyancy are significantly stronger than the velocities that weak swimming organisms can sustain. Hence, weak swimming or drifting organisms are prevented from sustaining all but the briefest duration of exposures to effluents within this allowable "zone of acute criteria exceedance". The rapid mixing itself provides substantial protection from acute toxicity. Only organisms capable of swimming strongly, such as a fish, could return to this turbulent area for repeat exposures. Such an organism could also avoid such an area. Attached organisms within the area of acute criteria exceedance could receive longer durations of exposure, but they are within the zone where that impact would be allowed. Benthic organisms within this zone are generally not exposed because the initial plume direction is upward.

The present draft mixing zone regulation allows for consideration of duration and frequency of exposure, although it doesn't specify how. Current permit writer guidance, however, simply ignores these issues entirely, leading to permit requirements such as whole effluent toxicity tests and toxicity reduction evaluations which are both expensive and, as implemented, irrelevant to water quality criteria in the receiving waters. Without specific guidance, the permit writers are unlikely to independently consider these factors. The responsibility will fall on the dischargers to present information specifically addressing this issue for the permit writer to be able to consider it at all. Otherwise, the discharger must simply accept whatever monitoring conditions the permit writer requires. In Washington, this typically includes acute biomonitoring studies with non-fish species that describe a sensitivity to an effluent, but have no bearing on acute toxicity in the receiving waters.

WASHINGTON STATE'S BIOMONITORING REQUIREMENTS

Major industries in Washington State have had an effluent toxicity limit in their permits based on a 96 hour salmonid acute bioassay. Dischargers were required to have less than 20% mortality in either 65% effluent or in 100% effluent. A few major municipal permits have had similar requirements and have allowed the samples to be dechlorinated before testing. These dischargers have shown that their discharges consistently meet this standard. Because the technology has shown it can meet these limits, DOE now considers them as "technology based" limits. Testing has been generally required either 4 times a year or 2 times a year. Any test that fails is considered a permit violation, subject to enforcement actions such as fines.

Upon failing a test, the dischargers would have to re-test every month for 3 consecutive months, passing all tests before they could revert back to their normal monitoring frequency.

In June 1988 DOE prepared an interim biomonitoring policy (DOE 1988). This described requirements for dischargers to perform acute bioassays with three different species to determine a most sensitive species. Initial testing would be six times a year for the first year, and after the first three tests, the number of species would drop to just the most sensitive species. After that, testing would use the most sensitive species and test four times a year. The interim biomonitoring policy provided no details concerning chronic bioassays. It did describe a method to determine a numeric value for toxicity which would be considered equal to the numeric values for acute criteria. The interim policy provided no means to consider the effective reduction in toxicity associated with duration of exposure.

In 1989, DOE started to issue draft permits for major industrial and municipal dischargers that included acute bioassay limits, plus a three species acute biomonitoring study with enforcement implications as per the June 1988 interim biomonitoring policy. These permits also included a requirement for three species chronic bioassay studies and even defined specific protocols to use. Acute toxicity assessment in those permits did not consider duration of exposure.

Following numerous critical comments from industry representatives and consultants, DOE hired a new employee to re-write the biomonitoring policy. That policy preparation is still underway at this time. Nevertheless, permits have already been issued. More recently, the state has been considering adopting in its water quality regulations a means for defining and numerically quantifying acute and chronic toxicity.

The draft biomonitoring policy provides for testing with three species for acute bioassays and determining the most sensitive. The most sensitive species then will continue as a permit monitoring requirement. If acute toxicity is determined to exist, following whatever dilution might be allowed by the mixing zone regulation for an individual discharger, then a discharger could be required to implement a Toxicity Identification Evaluation (TIE) and a Toxicity Reduction Evaluation (TRE) following EPA guidelines (EPA 1988a, b, c, d, e). Ultimately, a similar requirement could be imposed for chronic toxicity, but EPA hasn't completed its manuals for performing TIEs and TREs for chronic toxicity.

The monitoring costs alone associated with the draft biomonitoring policy are substantial. DOE estimates there are 10,000 dischargers that should be under NPDES permits. Excluding characterization costs, routine monitoring would likely be 4 chronic tests and 6 acute tests per year. Assuming $1,000 per chronic test and $200 per acute test, the monitoring costs alone will be $52,000,000 per year.

The draft biomonitoring policy does not provide any recognition of the benefit of rapid dilution which reduces the feasible duration of exposure for an organism in the receiving waters. Dr. Donald Mount of EPA's research lab at Duluth, Minnesota has been instrumental in developing biomonitoring techniques. In a deposition taken in 1989 Dr. Mount was critical of states using toxicity tests without considering concentrations in the receiving water and durations (Mount, 1989).

DURATION OF EXPOSURE MAY DETERMINE RELEVANCE OF TEST SPECIES

EPA's TSDs (1985 and 1990) acknowledge that duration of exposure and frequency of exposure are important. However, these documents do not offer any way to incorporate these considerations. The numerical values for acute toxicity, whether for individual chemicals or for whole effluent toxicity, are intended by EPA to be values that should not be exceeded for a duration of more than an hour every three years. For short-lived organisms, basically they should not receive an exposure of more than one hour to levels greater than acute criteria levels in their life time. However, the determination of acute toxicity is made by laboratory tests with exposures to constant concentrations for 48 hours or 96 hours depending on the test, and the real world exposures in close to turbulent plumes may be just seconds. Constant concentration exposures are not representative of real world mixing and are overly protective in themselves.

The effect of moving the zone of acute criteria exceedance closer to the outfall, such as would occur if a modern diffuser were added to an outfall, is to reduce the duration of exposure, and therefore reduce any acute toxic effect. Furthermore, such increased mixing would also reduce the duration of exposure to chronic levels further out in the mixing zone. This must be considered both in the tests themselves, and in the use of the test data for regulatory purposes (e.g., setting effluent limits).

For many dischargers, non swimming, or weak swimming organisms such as Daphnids or algae are not appropriate to base any permit limits for acute toxicity on unless it is also possible to adjust such a limit to recognize the benefit of the greatly reduced opportunity for prolonged exposure inherent in many combinations of ambient currents and diffuser designs. They may be more sensitive, but their exposure is less. In such cases, testing with fish species is appropriate, as a fish can return to the vicinity of a discharge for repeat exposures.

Consider now that a discharger may determine that it exceeds an acute toxicity value, either for a specific constituent or for toxicity itself, at the concentration predicted to occur at the edge of the zone of acute criteria exceedance. This could trigger a requirement to reduce "toxicity," (without regard to actual biological impact) with substantial costs for studies and treatment plant or process changes. Looking at Figure 3, it is possible to identify that the effluent in question would meet the criteria at some distance further out, and that such added distance may actually be quite small. The volume of water that toxicity reduction efforts would then be directed towards protecting would be just the area between the two arcs in Figure 3 in which a small portion of the plume may be exceeding acute criteria. The potential for expending substantial costs to accomplish a minute change of possibly no consequence whatsoever is great. This is especially true if the test that triggers a TRE is based on a species that is highly protected from a significant duration of exposure by both the smallness of the zone of acute criteria exceedance and the physical properties of effluent dilution and ambient currents.

A final consideration here. Assuming that a fish is the appropriate species to test acute toxicity the TSD provides a means for determining a concentration for which there is "no toxicity". It requires testing effluent dilutions to determine an LC50, and then multiplying that concentration by 0.3. The TSD says this will define an LC1, and that an LC1 is effectively "no toxicity". This approach itself may be overly protective for many combinations of test species and effluents. Lets assume for purposes of discussion that this approach is valid. Now, compare the derived LC1 concentration to the zone of acute criteria exceedance in EPA's TSD and the dilution attained at the edge of that zone. Also assume for purposes of discussion that this approach appropriately uses a fish species. Given these assumptions, it is apparent that for many dischargers, they are already meeting a far more stringent, technology based limit with their existing permit requirements for greater than 80% salmonid survival in either 65% or 100% effluent. The explanation for this follows:

1. Conservatively assume there is a dilution factor of 10 at the zone of acute criteria exceedance (based on EPA's statement for the majority of dischargers, whose zone of acute criteria is based on discharge port size).

2. This means that 10% effluent would have to meet an LC1 .

3. Working backwards from the LC1 to the LC50, 10% divided by 0.3 shows that 33% effluent would have to meet an LC50.

4. Present dischargers meeting an LC20 (80% survival) in 65% effluent are achieving at least a 30% greater survival

than required in effluent twice as concentrated as that they would have to meet to not cause acute toxicity at the edge of the zone of acute criteria exceedance.

5. **Present dischargers meeting an LC20 (80% survival) in 100% effluent are achieving at least a 30% greater survival than required in effluent three times as concentrated as that they would have to meet to not cause acute toxicity at the edge of the zone of acute criteria exceedance.**

Based on this analysis, there is no reason to require the acute biomonitoring studies in renewed discharge permits in Washington State for dischargers that are already subject to an effluent toxicity limit based on greater than 80% survival of salmonids in either 65% or 100% effluent. The existing limit is substantially more stringent than any limit that would be developed from the required studies assuming only fish species are appropriate for discharges where the plume and the currents limit the feasible duration of exposure for non fish species.

The DOE's interim biomonitoring policy utilized the same approach as the TSD for determining an LC1 (DOE 1988). The present draft biomonitoring policy (DOE 1990a) utilizes a different approach, but EPA is pressuring the state to be consistent with EPA. The different approach requires that a No Observed (acute) Effects Concentration (NOEC) be determined rather than a computed LC1. In practice, this NOEC and an LC1 would be similar and the conclusion of the previous paragraph remains the same.

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PLATFORM SESSION

Aspects of Bioavailability

Chair: A. Lewis

MERCURY LEVELS IN FISH FROM THE WILLISTON LAKE AREA OF NORTH CENTRAL BRITISH COLUMBIA, A MERCURIFEROUS ZONE. **T.A. Watson**, Triton Environmental Consultants Ltd., *120-13511 Commerce Parkway, Richmond, BC, Canada (604-279-2093)*; *O. Fleming, Environmental Resources,* British Columbia Hydro, 1312-808 Nelson St., Vancouver, BC, Canada; R.V. Bogdonov, Triton Environmental Consultants Ltd.; H.A. Smith, Environmental Resources, British Columbia Hydro.

Williston Lake, the largest freshwater body in British Columbia, was formed in 1968 by impoundment of the Peace River which is in the vicinity of the Pinchi Fault, an area naturally high in mercury. The influence of reservoir formation and high background levels of mercury were evaluated with respect to mercury levels in fish sampled from 1970 to 1990. Levels exceeding the Canadian guideline limit of 0.5 mg/kg were observed in several species from Pinchi Lake in the late 1960's and early 1970's. Mercury concentrations in piscivorous species, bull trout (*Salvelinus confluentes*) and burbot (*Lota lota*), sampled in 1979 and 1988 within the Williston Lake reservoir were higher than in other species. Levels in bull trout in 1979 ranged from 0.09-1.69 mg/kg and in 1988 from 0.018-4.87 mg/kg. Concentrations in burbot were generally lower, ranging from 0.013-0.68 mg/kg in 1988. Planktivorous species and those with mixed feeding habits such as rainbow trout (*Salmo gairdneri*), lake whitefish (*Coregonus clupeaformis*) and kokanee (*Oncorhynchus nerka*) seldom exceeded mercury levels of 0.5 mg/kg. Mercury levels were strongly correlated with weight for each species. Analysis of covariance indicated that high mercury levels and rates of uptake have not changed significantly in Williston Lake fish populations between 1979 and 1988.

MULTICOMPONENT KINETIC ANALYSIS OF IRON SPECIATION IN HUMIC LAKE TJEUKEMEER: COMPARISON OF FULVIC ACID FROM THE DRAINAGE BASIN AND LAKE WATER SAMPLES.

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Iron speciation in Lake Tjeukemeer, The Netherlands, was studied by multicomponent kinetic analysis of the ligand exchange reactions between TPTZ (2,4,6-*tri*(2-pyridyl)-*s*-triazine) and naturally occurring ligands bound to iron. Comparison with the kinetic behaviour of iron in synthetic solutions made of extracted fulvic acids from the Lake drainage basin indicates that iron seems to be distributed in two forms; polymeric hydrous oxides and, possibly, iron fulvates.

SUMMARY.

The importance of humic substances in the circulation of nutrients in humic lakes has been more often inferred than actually verified. It is a common practice to extrapolate results form laboratory studies involving isolated humic substances to natural water samples. Although laboratory studies are necessary in order to understand the main features of nutrient (i.e. Fe) interactions with humic substances, studies including actual samples are necessary. A careful comparison of both results will permit a better assessment of the importance of humic substances in nutrient circulation.

The ligand exchange reaction between TPTZ and naturally occurring ligands bound to iron ($Fe\text{-}L_i + C \longrightarrow P$) was followed spectrophotometrically by monitoring the formation of iron-TPTZ complex in fulvic acid solutions and lake water samples at 590 nm. The absorbance versus time plot were fitted to the Equation 1

$$P(t) = \sum_{i=1}^{n} A_i \left(1 - \exp(-k_i t) \right) + X \qquad (1)$$

where A_i in the initial concentration of each iron complex ($Fe\text{-}L_i$), k_i the rate of dissociation of each complex and X is the concentration of free iron and any other complexes which can react "instantaneously" with the complexing agent C after blank correction. Multicomponent kinetic analysis permits the deconvolution of the various first order rates comprising the observed overall dissociation rate.

The presence of fulvic acid in synthetic iron solutions changes the speciation of this metal. This is clearly seen in Table I. Comparison of the rate of dissociation of the iron-fulvic species with those of iron oxides indicates that a fraction of the iron-fulvate complexes dissociate faster that hydrous oxides. The fact that iron-fulvic complexes are more labile than hydrous oxides has already been demonstrated by Langford et al. (1977). Component A_2 for the solution containing fulvic acid has a rate of the same order of magnitude as that of the hydrous oxides. This probably implies that under the present experimental conditions, iron is distributed among iron-fulvates and hydrous oxide species.

These two iron species behave differently with respect to changes in pH. The variation of dissociation rate constants with changes in pH as equilibrium may give an indication of the level of heterogeneity of the species (Table I). Hydrous oxide seem to be a "well defined" species which could be modelled by a single dissociation rate constant at the ph range under consideration. On the other and, the decrease of the

Table I. Summary of dissociation rate constants for iron hydrous oxides and iron fulvates in laboratory solution

		Percent total iron			Rate (min^{-1})	
Sample	pH	$\underline{X}^{a}$ (8%)[d]	$\underline{A}_1{}^{b}$ (5%)[d]	$\underline{A}_2{}^{c}$ (6%)[d]	$\underline{k}_{A1}$ (10%)[d]	$\underline{k}_{A2}$ (10%)[d]
Fe^{+3}	3.00	74.1	25.9	—	0.02	—
Fe^{+3}	4.30	50.4	49.6	—	0.02	—
Fe^{+3}	6.23	26.0	74.0	—	0.01	—
Fe^{+3}-FA[e]	3.00	79.0	17.9	2.80	0.83	0.06
Fe^{+3}-FA[e]	6.02	13.2	66.5	20.2	0.22	0.04
Fe^{+3}-TjFA[f]	6.23	3.3	81.1	15.6	0.20	0.05

[a] "Instantaneous" component. [b] "Fast" component. [c] "slow" component. [d] Percent error. [e] Solution containing fulvic acid extracted from Lake Tjeukemeer's drainage basin. [f] Solution containing fulvic acid extracted from Lake Tjeukemeer.

dissociation rate constant with increasing pH for iron-fulvates suggests that more than one ligand is required for modelling this system. Furthermore, the experimentally obtained rate constants for iron-fulvates may represent average values for binding site distribution.

Examination of the results involving Lake Tjeukemeer water samples indicated that iron is present in at least two forms (Table II). As first approximation, each of thee forms have dissociation rates comparable to those of iron-fulvates and iron oxides. The rates corresponding to component A_1 are nevertheless significantly higher that their corresponding counterparts in fulvic acid solutions, although they all have the same order of magnitude. As in the case of fulvic acid solutions, the pH dependency of dissociation rate constants for iron-organic complexes are probably averages corresponding to a distribution of sites.

The lack of an "instantaneous" component in Lake water samples, suggests that all of the iron present in tied up in organic and inorganic complexes (colloidal and polymeric), which take between 1 minute to 1 hour to dissociate. In contrast, in a synthetic solution of Fe(III) only, approximately 26% of the iron is readily available (see Table I). This observation supports previous studies by De Haan et al. (1985) in which iron from Lake Tjeukemeer colloids was less available to algae that from $NH_4(FeSO_4)_2$ 12 H_2O solutions. In the latter case, Fe(III) may become available by oxidation of Fe(II) rather than from the dissociation of polymeric Fe(III) as in the case of synthetic solutions and natural organic colloids.

Another observation deserves some comments. Ultrafiltration of Lake Tjeukemeer water through a AC 64 S&S ultrafilter resulted in an apparent change in iron speciation. This change is reflected in a shift towards the "instantaneous" reactive form. An important practical consequence of this result in that ultrafiltration may change the speciation of iron within a size class. More experiments involving kinetic analysis of ultrafiltrates are necessary to further document this result.

The results of the present study provide support for the use of multicomponent kinetic analysis to determine iron speciation in natural water samples. This technique can be used to monitor changes in iron speciation due to seasonal variations and changes in water quality. No sample manipulation is required apart from the initial filtration step. The only previous report on the use of this technique in iron speciation in natural waters is that of Tipping et al. (1982). In their study they used sulfosalicylic acid (SSA) as the exchange ligand. The molar extinction coefficient of iron-SSA complex is of the order of 10^3 $M^{-1}cm^{-1}$, while that for iron-TPTZ complex, as determined in our laboratory is 2.1×10^4 $M^{-1}cm^{-1}$. So the use of TPTZ represents an improvement or one order of magnitude in the detection of iron. In principle, iron concentrations as low as 1×10^{-7} M could be

Table II. Summary of dissociation rate constants for iron in Lake Tjeukemeer water samples

		Percent total iron			Rate (min^{-1})	
Sample	pH	$\underline{X}$[a] (8%)[d]	$\underline{A}_1$[b] (5%)[d]	$\underline{A}_2$[c] (6%)[d]	$\underline{k}_{A1}$ (10%)[d]	$\underline{k}_{A2}$ (10%)[d]
TjW-0.2[e]	6.4	—	60.0	40.0	0.95	0.03
TjW-0.2[e]	6.4	—	63.6	36.4	0.92	0.03
TjW-AC64[f]	6.4	75.0	25.0	—	0.94	—
TjW-0.2[e]	7.21	—	62.5	37.5	0.26	0.02
TjW-0.2[e]	3.31	44.0	—	56.0	—	0.01
TjW-0.2[e]	1.82	76.0	—	24.0	—	0.01

[a] "Instantaneous" component. [b] "Fast" component. [c] "slow" component. [d] Percent error. [e] Lake Tjeukemeer water filtered through a 0.2 um filter. [f] Lake Tjeukemeer water filtered through a 64 nm S&S ultrafilter.

detected with a 1 cm path cell length. This detection limit could be lowered by increasing the cell path length.

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Environmental Forcing of Primary Production in a Tidally Energetic Fjord Subject to Mine Tailing Discharge

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The Island Copper Mine (ICM) on Vancouver Island, B.C. has been discharging upwards of 4 x 10^4 tons of mill tailings into Rupert and Holberg Inlets since the early 1970's. As part of its B.C. Pollution Control Board permit to do so, the ICM has maintained an environmental monitoring program. The variables monitored includes two indicators of phytoplankton production (Chla specific biomass and C14 uptake rate) and several environmental parameters upon which primary production is nominally dependent (i.e. seawater temperature (T), incident irradiance (I_o), and water column static stability (E)). The last parameter is a measure of the capacity for a water parcel to resist vertical displacement and in the absence of a more direct measure is an approximate indicator of the vertical mixing regime. It was calculated for the purpose by finite differencing density over the 0-5 m and the 5-20 m water column (E_T and E_B respectively).

A statistical analysis of these variables and parameters (Kessler and Parsons 1989a) implicated seasonal variations in near surface mixing in the environmental control of primary production (Table 1). The sign change between upper water column mixing potential and phytoplankton standing stock occurs at the time of a putative change in relative buoyancy of the tidal inflow (Stucchi 1985), thus implicating seasonal changes in the tidal flow in this relationship. The underlying mechanism could be a combination of direct dispersion of standing stock along concentration gradients and indirect enhancement or suppression of phytoplankton growth rate. However, the opposite sign of the B-E_B anti-correlation and P-E_T correlation is consistent with a dominance by the biomass dispersion effect (Table 1).

Inter-annual changes in water column light attenuance have been identified for Rupert and Holberg Inlets that coincide with changes in the bottom sediment re-suspension regime (ICM 1983). While a clear relationship between growth rate and light attenuance *per se* was not identified in the statistical analysis of the monitoring data, the coincidental variation in sediment re-suspension and light attenuance coupled with a strong correlation between growth rate and surface irradiance consistent with light-limited phytoplankton growth (Table 1), suggests that bottom scouring may be an

Table 1 *Partial correlations (with year and station fixed) between biomass ($B(z)$ and B^S) and productivity ($P(z)$ and P^I) and, irradiance (I_0) and water column stability (E_T and E_B). The parenthetical coefficients calculated with I_0 fixed as well. Dash indicates a non-significant correlation ($P>0.05$). Rows with no significant coefficients have been deleted. Integers in parentheses denote depth in meters.*

	April	May	June	July	Aug.	Sept.	Oct.
Holberg-Rupert basin							
E_T							
B(1)	-0.461	-	-	-	-	-	-
B(3)	-0.618	-	-	-	-	-	-
B(5)	-0.641	-	-	-	-	-	-0.603
B^S	-0.613	-	-	-	-	-	-0.550
$P(1)$	-	-0.568 (-)	-	-	-	0.456	-0.627
$P(3)$	-	-0.610	-	-0.474	-	-	-0.583
$P(5)$	-	-0.562 (-)	-	-	-	-	-
P^I	-	-	-	-	-	0.568	(-0.495)
E_B							
B(1)	-	-	0.620	0.615	-	-	-0.531
B(3)	-	-0.509	0.583	0.508	-	-	-0.449
B(5)	-	-0.501	0.399	-	-	-	-0.429
B^S	-	-0.424	0.671	0.507	-	-	-
$P(5)$	-	-	-	-	(-0.426)	-	-0.562
P^I	-	-	-	-	-	-	-0.571
I_0							
$P(1)$	-	0.590	-	0.531	-	-	-
$P(3)$	-	0.589	-	0.465	-	-	-
$P(5)$	-	0.632	0.589	-	0.406	-	-
P^I	-	0.593	-	-	-	-	0.429

additional mixing related process affecting primary production in Rupert and Holberg Inlets, albeit on a different time scale. Since the effect of the latter is likely to be influenced by mill tailings discharge (i.e. an additional source of unconsolidated bottom sediments), quantifying the relative importance of the two is germane to an assessment of tailing discharge effect on primary production. Partly with this in mind, a field investigation was undertaken in Holberg Inlet in 1982. In essence the study consisted of repeatedly sampling a parcel of surface water to obtain a time series of water properties.

The salient features resolved were the evolution of a subsurface maximum of phytoplankton biomass (SCM) that descended from an initial depth of 2 m to a final depth of 3 m over a 3 day period (Figure 1). The SCM remained fixed at 3 m depth for the remaining two days of the time series. Specific phytoplankton growth rates required for the observed increase in biomass and depth dependent differences in the species assemblage are independent lines of evidence indicating that a behavioral aggregation response by motile phytoplankton is at least a partial contributor to the SCM formation (Kessler and Parsons 1989b). The carbon uptake rate profile lagged behind SCM development by several days, with the depth of peak growth rate not reaching 3 m depth until the sixth day (Figure 1). There was some suggestion that the evolving growth rate profile was controlled by a descending nitracline though this inference was equivocal due to a water column uplifting event (Figure 1). The depth distribution of growth rate was not measured after the sixth day. However, an indirect estimate of biomass distribution (i.e. *in vivo* Chla fluorescence) on the ninth day (Figure 2) indicated an essentially unchanged SCM depth and by inference a depth of maximum growth rate well above the 1% light depth of about 15 m.

The time series lends itself to a mass balance analysis, allowing internally consistent estimates for the various source/sinks terms to be made, including diffusive exchange. Since these estimates are clearly dependent on one another, the calculation was organized such that empirically based terms or those for which a consensus has emerged in the literature were calculated first. Terms that are poorly constrained, either because this consensus is lacking or because they are geographically dependent, were calculated last. Some independent verification of the calculation does accrue from the simultaneous balancing of the two budgets.

The change in Chla biomass integrated over the top 6 m of the water column (dChla) was predicted by a light-limited growth model to be 161 mg m^{-2} while the measured dChla was only 15 mg m^{-2}. Similarly, dNO_3^- was predicted to be -460 mg m^{-2} whereas the

FIGURE 1

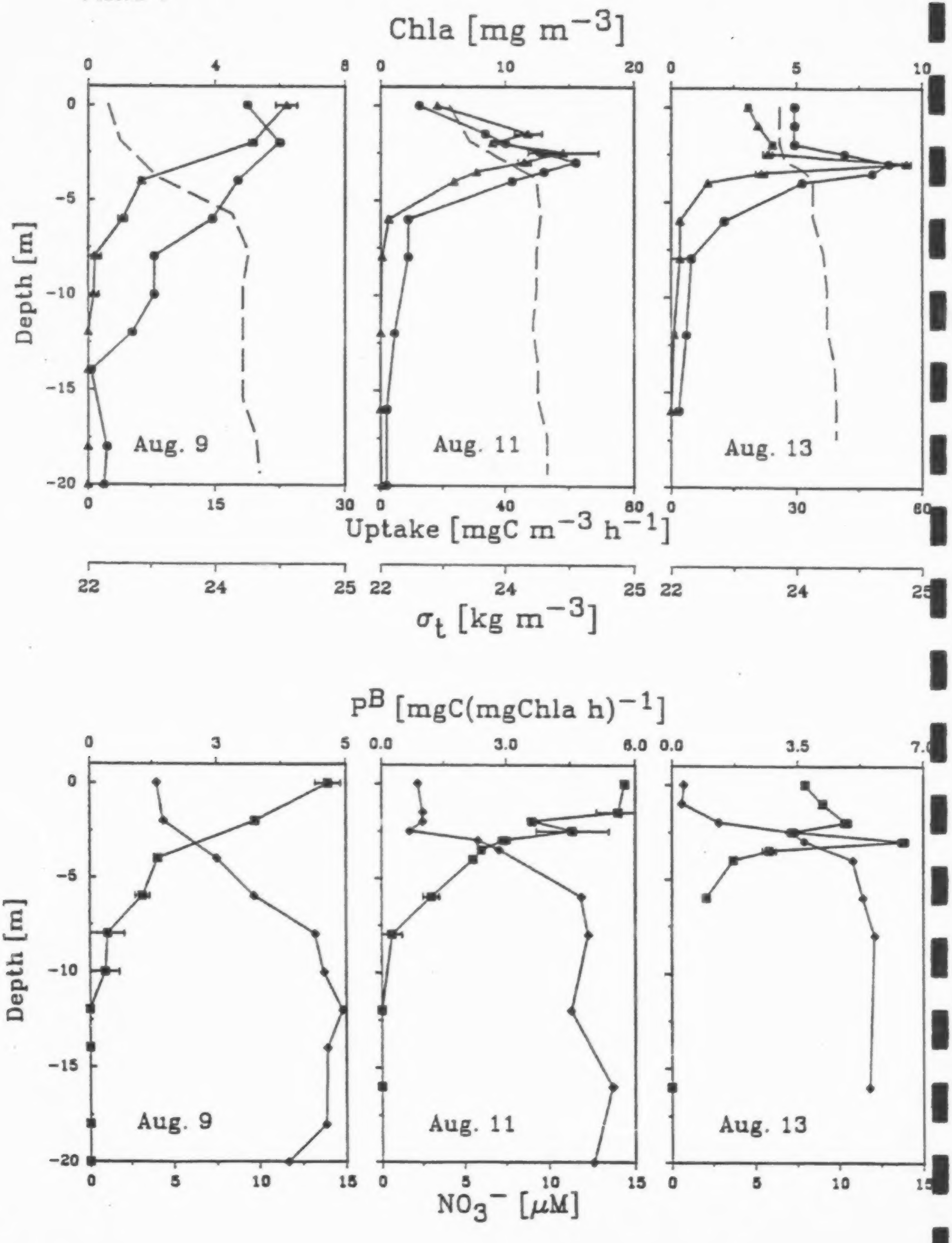
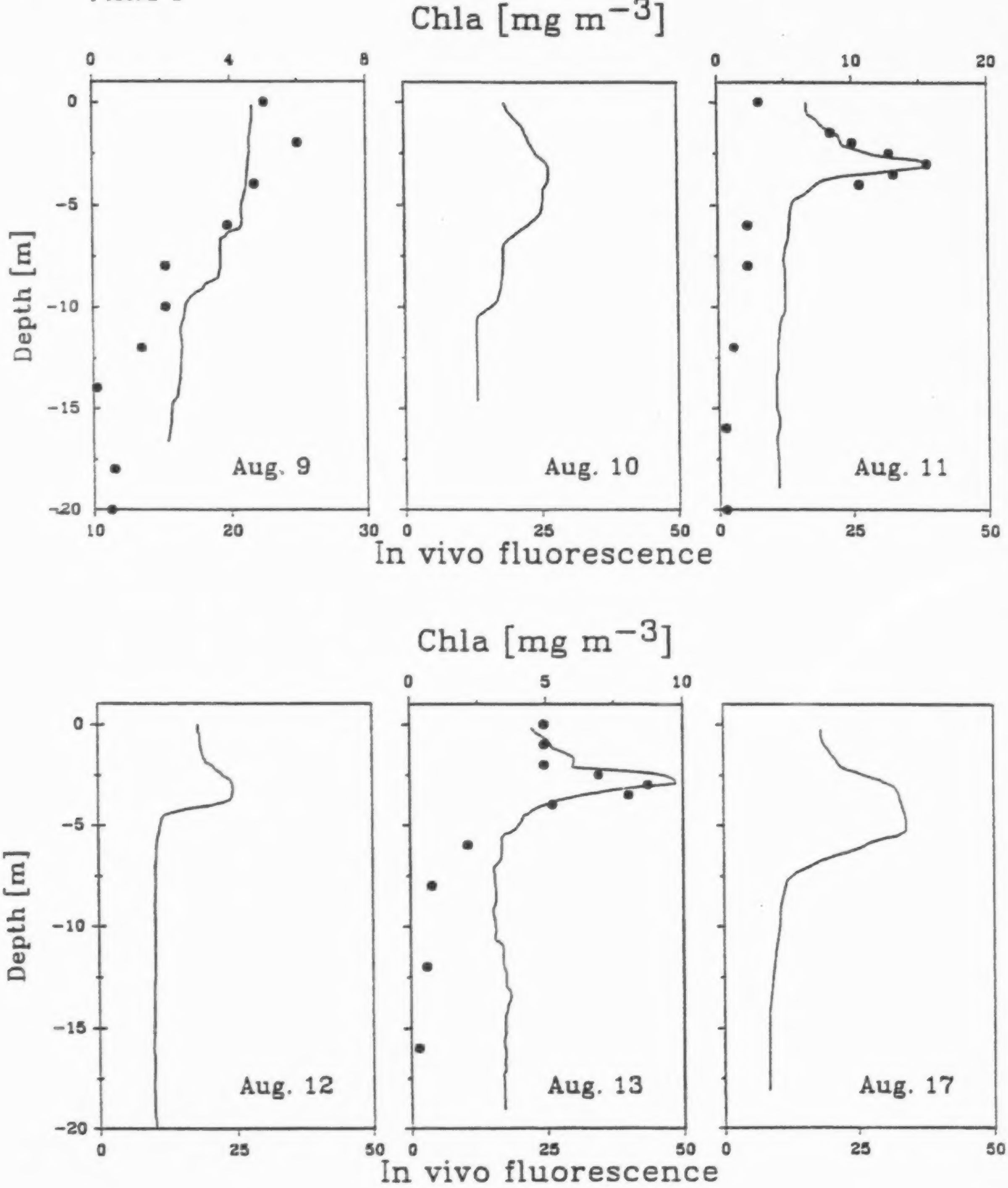

Chla [mg m^{-3}]
0
4
8
0
10
20
0
5
10
Depth [m]
0
-5
-10
-15
-20
Aug. 9
Aug. 11
Aug. 13
0
15
30
0
40
80
0
30
60
Uptake [mgC m^{-3} h^{-1}]
22
24
25
22
24
25
22
24
25
σ_t [kg m^{-3}]
P^B [mgC(mgChla h)$^{-1}$]
0
3
5
0.0
3.0
6.0
0.0
3.5
7.0
Depth [m]
0
-5
-10
-15
-20
Aug. 9
Aug. 11
Aug. 13
0
5
10
15
0
5
10
15
0
5
10
15
NO$_3^-$ [μM]

FIGURE 2


Chla [mg m^{-3}]
0
4
8
0
10
20
Depth [m]
0
-5
-10
-15
-20
Aug. 9
Aug. 10
Aug. 11
10
20
30
0
25
50
0
25
50
In vivo fluorescence
Chla [mg m^{-3}]
0
5
10
Depth [m]
0
-5
-10
-15
-20
Aug. 12
Aug. 13
Aug. 17
0
25
50
0
25
50
0
25
50
In vivo fluorescence

observed value was -170 mg m^{-2} (Table 2). Regenerated nitrogen supports about 60% of the total measured primary productivity in productive coastal waters. Assuming a similar component for Holberg Inlet reduces the NO_3^- defect of 290 mg m^{-2} by a factor of about two (Table 3). Similarly, cell size dependent sinking rates reported in the literature for nutritionally replete phytoplankton would serve to reduce the Chla defect by 30% to about 115 mg m^{-2}. Including a micro-heterotrophic grazing component, indirectly estimated from measured phaeopigment levels during the study would serve to reduce the Chla defect by a further 5%. Invoking all these additional terms brings dChla to within a factor of two to three of dNO_3^-.

The remaining divergences, along with the measured vertical gradients in Chla and NO_3^- can be used to estimate an internally consistent diapycnal mixing rate k_z. A calculation using the annual reduction in midwater column salt content in Holberg Inlet gave an upper estimate for k_z of about 10^{-4} m^{-2} s^{-1}. Invoking this mixing rate accounts for 80-90% of the missing Chla, but is a factor of 50 greater than the diffusivity required to balance NO_3^-. By the same token, a magnitude for k_z of 4×10^{-6} m^{-2} s^{-1} that balances the NO_3^- can account for only about 3% of the missing Chla. However, the latter it is within a factor of two of a theoretical relationship between internal wave dissipation and vertical diffusion, calibrated by data from a sill-fjord (Svensson 1979). An internal wave dissipation energy source is consistent with the persistent pycnocline at SCM depth during the study (Figure 1).

Adopting the smaller diffusion rate leaves a large defect in the observed dChla. While no nighttime measurements of grazers was undertaken during the study, qualitative 3 m depth net tows in Holberg Inlet one month later in September 1982 (ICM 1982) indicated a nighttime community dominated by large herbivores with clearance rates three to >30 times higher than the daytime grazers observed during the study and could in moderate densities provide the necessary factor of 10^3 greater grazing pressure necessary to account for the missing biomass. Since by definition these grazers would be vertical migrators, their contribution to ambient nitrogen levels would be correspondingly small thus minimizing the associated imbalance of the NO^-_3 budget.

Conclusion

The consequence of elevated light attenuance can be put in some perspective on the assumption that the observed vertical distribution of water column properties and subsequent biomass and nutrient budget calculations are representative of summertime

Table 2 *The 0-4 m Chla and NO_3^- mass balance for drift station A1, with nitrogen demand and phytoplankton growth calculated from phytoplankton carbon fixation rates after losses and gains due to daytime grazing respectively. δNO_3^- and δChla are the measured NO_3^- disappearance and Chla increase at A1.*

Date	N-demand [mgN m^{-2}]	δNO_3^- [mg m^{-2}]	Growth [mgChla m^{-2}]	δChla [mg m^{-2}]
Aug. 9	70	-	24	-
10	85	-	30	-
11	99	361	35	19
12	139	-	49	-
13	66*	−211	23	−16(−4)†
Total	459	150	161	3 (15)

* production prior to sampling time

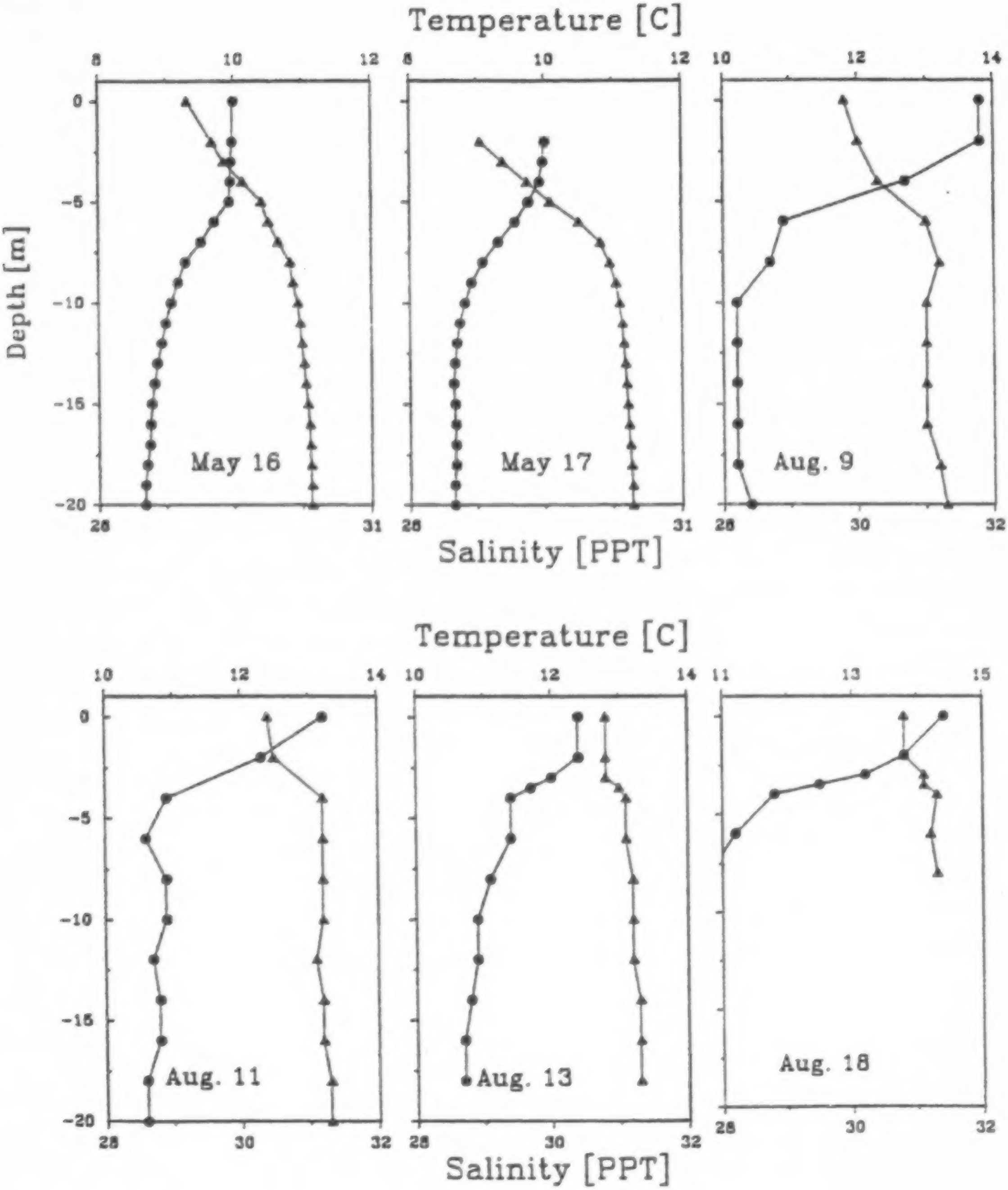
† Based on change in SCM *in vivo* fluorescence

Table 3 *The calculated sink and source terms which reconcile the divergence between the drift station A1 Chla and NO_3^- mass balances. Mass defect is the difference between the observed change in mass and that predicted from measured phytoplankton growth rate. Specific nitrogen remineralization terms (e.g. macro-zooplankton daytime grazing) are assumed to enter a reduced nitrogen pool and hence not contribute to the NO_3^- mass balance calculation.*

Budget Term	NO_3^- [mgNO_3^- m^{-2}]	Chla [mgChla m^{-2}]
mass defects from *Table*	309	158(146)*
macro-zooplankton daytime grazing	-	0.3
microheterotroph grazing	-	8
sedimentation	-	44
40% N recycling	184	-
advection	23	2
outstanding defect	102	103(92)

*based on *in vivo* fluorescence Aug. 13 biomass

FIGURE 3


Temperature [C]
8
10
12
8
10
12
10
12
14
Depth [m]
0
-5
-10
-15
-20
May 16
May 17
Aug. 9
28
31
28
31
28
30
32
Salinity [PPT]
Temperature [C]
10
12
14
10
12
14
11
13
15
0
-5
-10
-15
-20
Aug. 11
Aug. 13
Aug. 18
28
30
32
28
30
32
28
30
32
Salinity [PPT]

conditions. While the strong correlation between surface irradiance and phytoplankton growth rate in the monitoring dataset is consistent with light limited growth, the steep nutrient gradient in the pycnocline suggests that this may be an over-simplification. As surface irradiance decreases the depth of maximum growth shoals in response to the reduced light level at depth. However, this adjustment occurs within a steep ambient nutrient gradient, and therefore must reflect a balance between the increasing light flux and concomitant decreasing nutrient flux. This opposition in gradients should result in a reduced range in the compensatory response to increased light attenuance otherwise expected and thereby acts to further decrease growth rate.

The steep nutrient gradient also suggests a possible reason for the dominance of the biomass dispersion effect over growth rate during summer and seasonality inferred from the monitoring data. An increase in vertical mixing resulting from a more buoyant tidal inflow would increase the diffusive loss rate of phytoplankton. It would also increase input of nutrient into the surface layer. However, the added nutrient could only be utilized by a shoaling growth profile. The predominant phytoplankton species during the Holberg Inlet study showed an apparent preference for submaximal light levels. Ignoring adjustments in the species assemblage, increased light attenuance during times of elevated surface mixing should therefore serve to increase primary productivity by allowing maximal near-surface nutrient utilization of these low light preference phytoplankters. Lower surface light levels in the spring/fall may have the same effect, which could explain the B-E_B sign reversal in the monitoring data.

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A COMPARISON OF TWO METHODS FOR DETERMINING METAL PARTITIONING IN OXIDIZED SEDIMENTS. M. C. Dutton, L. I. Bendell Young and F. R. Pick, Dept. of Biology, Univ. of Ottawa, Ottawa, Ont. Canada (613-564-2248).

A simultaneous (SIM) and sequential (SEQ) extraction procedure were compared. Sediments from 2 marshes were sampled in replicate and trace metals (Fe, Mn, Zn, Cu, and Cd) extracted into easily reducible, reducible and organically bound sediment components. When expressed as % of total metal recovered, both methods showed the same distribution for Cu and Fe among the 3 components, although twice the amount of organically bound Cu and Fe (ug/g) was removed by the SEQ as compared to the SIM method. In contrast, the SEQ method recovered less Mn (ug/g) from all 3 fractions as compared to the SIM method. However, as with Fe and Cu the distribution of Mn was the same for both methods. In general, there was good agreement between the 2 methods for Zn and Cd. Pretreatment of sediments with reducing agents, as is done by the SEQ method, may result in the increased extraction of both Cu and Fe from the organic sediment component. Advantages of the SIM over the SEQ extraction include, rapid sample processing plus minimal sample manipulation. Severe contamination problems (i.e. Cd and Cu) which were found with the first two reagents of the SEQ method can be eliminated with the use of a SIM extraction. Therefore for the partitioning of metals in the above three fractions we recommend the use of a SIM extraction.

EVALUATION OF THE MAJOR PHYSICO-CHEMICAL PARAMETERS: THE DEGRADATION OF DDT TO DDE IN THE MARINE SEDIMENTS. A. Sarkar. Chemical Oceanography Division, National Institute of Oceanography. Dona Paula, Goa-403004, India. Telefax 0832-4612; Telephone No. 0832-6253.

In order to assess the environmental impact on the quality of the marine sediments, an attempt has been made to evaluate the major physico-chemical parameters influencing the degradation of DDT to its metabolite, Dichloro Diphenyl Ethylene (DDE) in the marine sediments. Altogether, 13 physico-chemical parameters such as pH, salinity, total organic carbon, metal ions like Al, Fe, Ti, Mn, Zn, Cu, Ni, conc. clay minerals, Bentonite, Ellite, and Kaolinite conc. have been considered for a stepwise multiple regression analysis with respect to DDE percentage. The best fitted multiple correlation ($R^2=0.962$) was obtained between DDE % and the parameters as follows:

$$\text{DDE \%} = -46.03 + 11.52\,\text{pH} - 0.46\,\text{Salinity} + 2.73\,\text{Fe} + 0.06\,\text{Zn} - 0.08\,\text{Cu} + 0.08\,\text{Ni} - 0.41\,\text{Ellite} - 0.17\,\text{Kaolinite}$$

PLATFORM SESSION

Relevance of Scandinavian Studies with BKME

Chair: P. Hodson

CONTRASTING FINDINGS FROM SCANDINAVIA AND NORTH AMERICA ON TOXICITY OF BKME. INTRODUCTORY COMMENTS.

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ABSTRACT. Concern about organochlorines in bleached kraft mill effluent (BKME) was generated in 1987 by Nordic research, especially in the Baltic. That indicated sublethal effects at 0.1% BKME while N. American research had seldom shown effects below 1% and sometimes not below 10%. Findings from both regions appear valid, and the following factors probably contribute to differences. (1) Nordic studies had strong components of field work and biochemistry. (2) Other toxicants in the Baltic almost certainly acted simultaneously. (3) Swedish mills lacked the secondary treatment common in U.S. mills. (4) The Swedish program focused on a mill in start-up mode.

Some past work has shortcomings, particularly for implicating organochlorines. (1) Comparison with an unbleached "control" effluent was often lacking. (2) Results were not presented in peer-refereed journals. (3) Smoothed average values were presented without supporting data. (4) Within-organism changes were not tied to whole-organism damage. Recent studies show greater trans-Atlantic overlap, for example papers in this session.

BACKGROUND ON SCANDINAVIAN STUDIES

This paper provides a background on the Scandinavian information that focussed attention on organochlorines in kraft mill effluent in 1987, and on the apparent contrast with North American work. The background is apparently not completely familiar to all workers in North America, so this paper sets the scene for others in this session which deal with more recent and particular research.

Concern about organochlorines in bleached kraft mill effluent (BKME) and paper products swept Canada in autumn of 1987 and has continued. The condemnation rested largely on Scandinavian findings, particularly the Swedish research program *Environment/Cellulose* (1983-85), reported at a 1987 conference in Tampere, Finland. This Swedish study around a BK mill on the Baltic Sea (Fig. 1), and a mill with no bleaching, involved a wide range of biological and chemical approaches in the laboratory, field, and in artificial ecosystems.

The conclusion was that effects of BKME were found at 10 km distance, at dilutions of 1/1000, i.e. 0.1% effluent. This was largely based on changes in: fish numbers, species, age and size near the mill; poor maturation of fish; fin erosion; growth of fish and number of invertebrates in artificial ecosystems; enlarged liver and induction of mixed-function oxidases (MFO); abnormal carbohydrate metabolism; and depressed number of white blood cells.

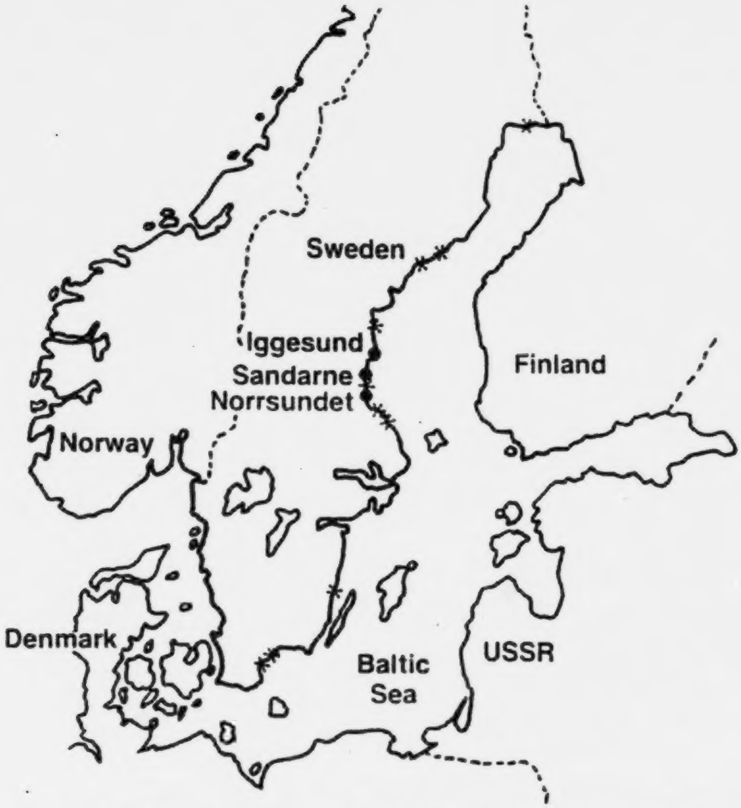

Fig. 1. Map of the Baltic Sea area with names and locations of three pulp mills used in the Swedish toxicological studies. Ten crosses mark locations of other Swedish mills on the Baltic. From Södergren (1989).

Near the BK mill, fish populations were apparently affected both by enrichment of the water and toxicity. Fish were virtually absent at 1 km distance, but at 2 - 3 km, there was an abundance of small, shallow-water species. Populations of larger, deeper-water fish were depressed for 4 km (Neuman and Karås 1987). Fish showed fin erosion up to 4 km from the mill, but at the unbleached mill no such fin erosion was seen, even at 100 m from the outfall (Södergren 1987).

Sexual maturation was delayed or depressed near the bleached kraft mill. At 2 km distance, only about 50% of potentially mature female perch had gonads developing, compared to 80% at great distance and at a control location (Sandström et al. 1987). Even at 10 km from the mill, the gonads were smaller in relation to body size (Larsson et al. 1988). Perch did not show such a problem near the unbleached mill. However, the perch grew faster near the BK mill than away from it, again a phenomenon that was not seen at the unbleached mill. Sandström et al. (1987) felt, from other work, that toxicants had disturbed the allocation of energy by the fish.

Evidence of such disturbed body chemistry was provided by a whole suite of biochemical/physiological tests on perch (Larsson et al. 1988). Effects were strong near the mill, but some of them continued to a lesser degree to 8 - 10 km distance. Limited studies near the unbleached mill showed lesser effects on most physiological parameters.

Extractable organic chlorine (EOCl) in perch was 200 - 400 mg/kg (fat weight), and gradually declined to a background level of 50 - 120 mg/kg at 11 km from the mill. Near the unbleached mill, EOCl was only 12 - 70 mg/kg (Södergren et al. 1987). At distant locations (18 km) there was still appreciable EOCl, but the substances present did not at all match those of BKME; either they were from other sources or had been transformed (de Sousa et al. 1987). [Recent budgets from Sweden indicate that somewhat less than 50% of adsorbable organic chlorine (AOX) input to the Baltic comes directly from pulp mills (Enell and Wennberg 1990)].

Artificial ecosystems simulated the community along the shores of the Baltic. Adding bleached kraft mill effluent at 1% caused tissue damage to the gills and livers of flounders in the systems, and increased the numbers of parasites on the gills (Lehtinen et al. 1984). Similar studies (SSVL 1985, Lehtinen 1989) showed that five kinds of kraft effluent caused meaningful sublethal effects on fish growth and populations of invertebrates at 0.25% BKME (1 in 400 dilution). The effects were most severe for the effluent from conventional bleaching and decreased for those with oxygen delignification, higher chlorine dioxide substitution, or secondary waste treatment. At 0.05% effluent (1 in 2000 dilution) the conventionally-bleached effluent caused growth reduction in sticklebacks, even after it had received treatment in an aerated lagoon (Lehtinen 1989).

FINDINGS IN NORTH AMERICA

Historically, North American toxicological research had not demonstrated appreciable differences in toxicity of bleached and unbleached effluents. Reviews and interpretations (McLeay 1987, Bonsor et al. 1988) provided generalizations on sublethal no-observed-effect concentrations (NOEC):

treated BKME	≥ 1%, often 10%
untreated BKME	≥ 0.5%, usually > 1%

Some of the most meaningful North American work has been done by the industry-supported National Council of the Paper Industry for Air and Stream Improvement, Inc. (NCASI), using fish in artificial streams. The research has also provided some of the highest NOECs in the literature, of 9% and 18% for growth and reproductive performance of fish exposed to well-treated effluent (Borton 1985). I am using the results from NCASI studies as the major comparison with Scandinavian research.

POSSIBLE REASONS FOR TRANSATLANTIC DIFFERENCES

The following four items would appear to be possible primary causes of different conclusions reached by researchers in Scandinavian and North American (N.A.), up to about 1988 (Sprague and Colodey 1989).

(1) Nordic studies were very strong on field research and biochemical appraisal.
(2) Other toxicants in the Baltic almost certainly acted simultaneously.
(3) Swedish mills lacked secondary treatment but U.S. effluents were often well treated.
(4) The Swedish program focused on a mill which was in start-up mode.

(1) Intensive and searching Nordic research effort.

The spectrum of Scandinavian toxicity work turned up some sensitive indications of biological change, for things that were not being studied in N.A. to any great extent. The Scandinavians placed great emphasis on field work as part of toxicity studies, not only population surveys, but growth, maturation, and biochemistry/physiology. The concerted interdisciplinary field studies of the Scandinavians are not common recently in North America, although they are not unknown (e.g. an issue of a Canadian journal filled with such a study (Kelso et al. 1977).

The Nordic effort was particularly strong on biochemistry and physiology for wild and caged fish. Sensitive techniques were used to assess defence enzymes, biochemical dysfunction, liver metabolites, as well as the more classical parameters of blood cells. Swedish workers did these in the Baltic Sea and in the laboratory (review by Andersson 1987), while the Finns also studied freshwater organisms (e.g. Oikari and Kunnamo-Ojala 1987). These components were rare or absent in North American studies of BKME effects. As described in other papers of this session, recent work shows that fish at some N.A. locations have "Scandinavian symptoms".

(2) Other toxicants in the Baltic acting in concert.

"*The Baltic is a seriously polluted area.*" (Olsson 1987)

The Baltic Sea is very sensitive to pollution. Although it is 1500 km in total length, it is shallow and has slow exchange (10 - 30 years residence-time). There is little tide, and because of a sill at the entrance, little entrance of deep oceanic water. The water is cool, resulting in slow biological turnover. It is heavily industrialized, receiving wastes from 5 countries.

Baltic birds, mammals and fish are high in bioaccumulative toxicants, for example the classic contaminants PCBs and DDTs. This is shown by residues in the guillemot, a colonial fish-eating bird related to the auks (family Alcidae), and an ideal species for use as a biological indicator. Residues in their eggs built up in the 1960s, peaked about 1970, but were still elevated in the early 1980s (Fig. 2). (Values for 1940 and 1955 were calculated from extant seal oil, assuming constant proportions between the bird and mammals.) The vertical lines in Fig. 1 have the following significance. A: The reproductive rate started to decrease among Baltic white-tailed sea eagles. B: Reproductive failure was discovered among the eagles. C: Mink population levelled off after a period of continuous increase following their escape into Swedish ecosystems. D: Failure reported in seal reproduction (Olsson 1987).

Contaminants continued to be present during Swedish pulp mill research. In 1987, the National Environmental Protection Board (NEPB) reported that "levels of DDT (DDE) and PCB in certain Baltic Sea organisms have begun to increase again after many years of decrease". Cod liver from the Baltic "contains so high levels of organic poisons that the [Swedish] National Food Administration has prescribed limitations for consumption" (NEPB 1987). PCB in Baltic animals "remains among the highest in the world regarding marine areas" (Jansson et al. 1987). Other, persistent contaminants, less easily measured, are present: "chlordane compounds, chlorinated terpenes, dibenzofurans, dioxins, chlorinated naphthalenes, PAH, HCB, etc." (Olsson 1987).

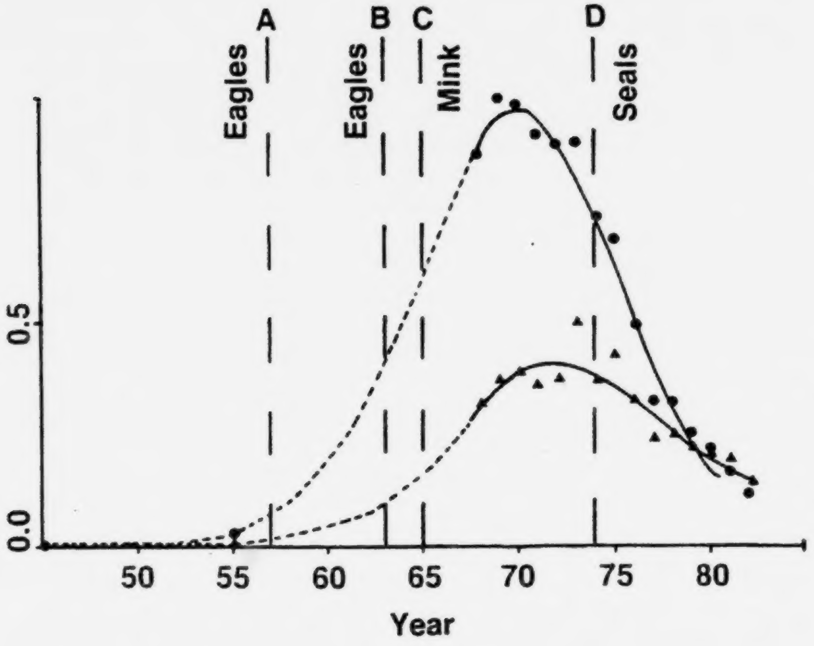

Fig. 2. Total DDT residues (ΣDDT, upper line) and ΣPCB (lower line) in the eggs of guillemot from the Baltic sea. Vertical lines mark times of reproductive or population crises in other fish-eating animals of the area. From Olsson (1987).

Problems with birds and mammals are serious indeed; apparently close to extinction are Baltic white-tailed sea eagles, and Baltic seals. The reproductive success of the Baltic eagles, at 0.3 young per pair, is among the lowest of 12 locations in the world for similar members of the genus, and may be compared with a rate of 1.1 - 1.4 young in Greenland and Alaska.

Baltic ringed seals are now rare along Swedish coast, although before 1940, >10,000 were hunted annually. Grey seals today number only about 1500 compared to several hundred thousand in the early 1900s. Harbour seals are represented today by 200 individuals, whereas they were previously found along most of the Baltic shoreline. The explanation is not hunting of seals, which is rare nowadays, and disturbance is apparently not a major factor. Fishing nets may kill 20% of young, not enough by itself to cause the population decline.

Among seals, there has been a "disease complex" since the 1970s with the following symptoms.

- Skin and claw lesions
- Skeletal abnormalities
- Abnormalities of uterus
- Intestine and kidney injuries
- Low reproductive rates

Toxicant accumulation is the probable cause of these problems. Baltic seals are high in many residues, by world standards. In particular, a suggested explanation is halogenated compounds acting on the endocrine system (see Appendix for further detail on this topic).

It is conceivable that all these effects on predatory animals might be caused by pulp mill effluent. More likely they arise from the combined action of the many toxicants. The corollary is that it may not be correct to conclude that all biological effects in the program Environment/Cellulose

- were caused by the BKME acting alone,
- at 10 km distance from the Baltic mill,
- at an effluent concentration of 0.1%.

If the effects in the Baltic represented combined action of many pollutants, it is easier to understand why North American toxicity studies carried out in otherwise clean dilution water, obtained higher values for the NOEC.

(3) Secondary treatment of effluent in U.S. but not in Sweden.

This could account for another major part of apparent differences in findings. Much of N.A. research showing high NOEC is from the U.S., especially the NCASI work. Essentially all U.S. kraft mills have secondary waste treatment, and the recent toxicity research has been done on treated effluent. For example, the NCASI research in their southern artificial stream used effluent which had been given 12-14 days of treatment including an aerated lagoon, in that warm climate (Borton 1985).

The main Swedish mill studied in 1983-85 (Norrsundet) had no secondary treatment.

(4) Swedish mill studied in 1983-85 was undergoing start-up.

In the first year of the Swedish program Environment/Cellulose, the mill reported a very high consumption of chlorine during start-up following modification, 59 kg chlorine/tonne of pulp produced. By 1985, consumption was only 31 kg/t (Neuman and Karås 1988).

Fin erosion was found in the first year, but not later. This may be taken as evidence that the 1983 results may not be relevant to low-bleach situations. Since 1985, chlorine consumption has dropped further, as reported in this session by Dr. Owens.

[This same information is ***in agreement with an interpretation that high consumption of chlorine in a kraft mill does increase the toxic effect of the effluent***, presumably because of the higher discharge of organochlorines.]

RECURRENT PROBLEMS IN SCIENTIFIC PROCEDURE

Toxicological and pollutional research in all countries, on both sides of the Atlantic, has sometimes had design or procedural problems that make results difficult to interpret. Often these relate to the difficulty of incorporating proper scientific design in a field project, or of obtaining sufficient funding for a large-scale field project.

(1) No comparison with unbleached effluent.

To document effects of *bleached* KME, it is necessary to have a parallel assessment of *unbleached* effluent. Sometimes that comparison was not included in the Swedish work, or not published. For example, mortality rates in perch populations were not given at the unbleached mill. Similarly, Larsson et al. (1988) found physiological disturbance in fish near the bleached mill but made the following general statement "... a limited investigation on perch caught in the receiving body of water of a pulp mill without bleaching processes showed no or considerably lower effects on most physiological parameters ..." but the details were not published.

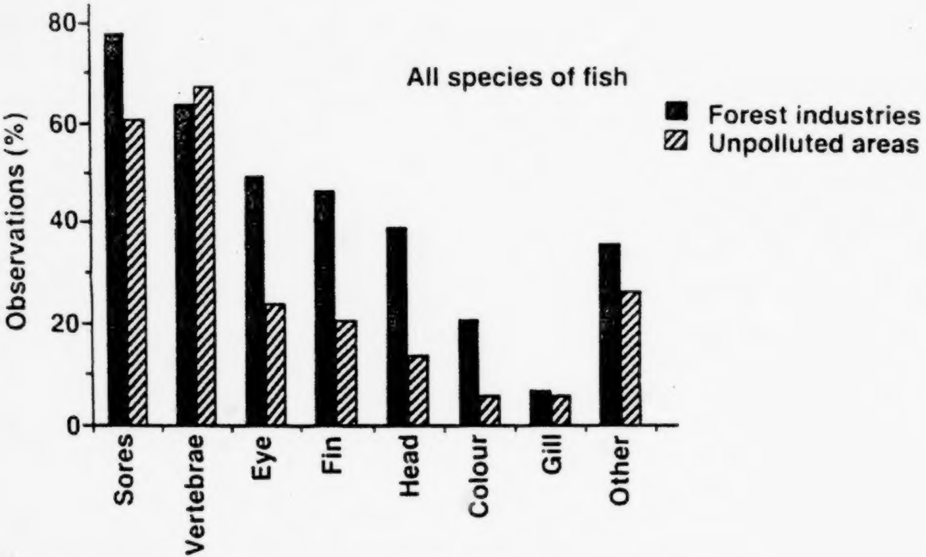

Fig. 3. Tabulation of abnormalities in Baltic fish. From Södergren (1989).

An example of the difficulty in assigning a toxic role to organochlorines from kraft mills is shown in Fig. 3, taken from a review of the Swedish work in the Baltic (Södergren 1989). Some of the differences are not large, between fish captured near pulp mills and those from "normal" control areas, and indeed there is one case of more abnormalities in a "clean" area. In such a set of data, it might be very difficult or impossible to differentiate between the degree of toxic effect from BKME and an unbleached effluent.

Virtually none of the N.A. lab tests on BKME have a comparison with unbleached KME. Some of the key U.S. research in artificial streams also lacked simultaneous comparison of different concentrations. For logistical reasons, different concentrations were run in different years.

From such experiments, one can see effects from the BKME compared to a control, but there is no way of ascertaining whether chlorinated substances were largely or partially responsible for the effects. One can compare the findings with those from other experiments using unbleached KME, but that is usually inconclusive because of inter-lab or inter-test variation.

(2) Some findings are not available in peer-refereed journals.

Some of the Scandinavian findings have been presented only as conference proceedings, as summaries, or in non-refereed reports. That was especially true up to about 1988, and since then more of the work has been finding its way to refereed journals (e.g. Lehtinen (1989), for tests done under the Swedish program in 1982-84).

In North America, there are similar deficiencies in presenting work in refereed publications. Findings are often presented in manuscript reports or in a series produced by an agency, institution, or commercial organization. In such cases the basic data are often presented for the reader but interpretation and formal analysis may be lacking.

A refereeing system may not guarantee that published work is first class, but it tends to have a salutary effect on presentation and analysis of data and conclusions drawn. Deficiencies in scientific presentation combine with the following item to create problems in evaluation.

(3) Supporting data not always available for independent analysis

Some fairly major and often-cited Scandinavian findings were not supported by published details needed by an outside observer to evaluate the conclusions. Part of the problem was related to the presentation of findings in short conference proceedings or summaries.

For example the failure of fish to mature near a bleaching mill, and their faster growth, was difficult to assess or relate to other places because the measured values were not given. For readers to compare the findings with their own, they need a presentation of fish ages and weights, plus gonad weights, not just the ratios of gonad to body weight. (See Owens paper, this session.) As another example, the levels of microsomal defence enzyme (EROD) in livers of Baltic fish were presented as a histogram of seasonal averages, whereas readers would probably wish to see also, the numerical values by season in order to evaluate variation with time, or among individuals or size-classes.

NCASI studies are generally good in providing details of findings. Reports may be thick and difficult to read but the raw data are given in profusion.

(4) Within-organism measurements not tied to meaningful effects

This criticism applies mainly to some studies of biochemical or physiological changes within fish. Recently in Canada, there have been reports of biochemical changes in fish "hundreds of kilometres downstream of pulp mills in the X river", without any indication of whether this was actually deleterious to the fish.

As has been said many times, simply finding a biochemical change within the fish does not necessarily mean that there is a deleterious effect on performance or viability of the fish, nor in this case, does it necessarily mean that organochlorines are involved. For example the induction of MFO in the liver of fishes, as measured by activity of EROD (7-ethoxyresorufin-o-deethylase), appears to be an extremely useful indicator of exposure to pulp mill effluent. It seems to interfere with reproduction of fish, or to be associated with such interference. It is, however, a generalized response (apparent defence mechanism) to many toxicants or wastewaters including municipal sewage, resin acids but not chlorinated phenolics, and leachate from wood chips (Fleming et al. 1990, Mather-Mihaich and DiGiulio 1989).

If the internal changes are measured as part of a large program, they can be tied to detrimental effects at the level of the whole organism, population, or community (e.g. Environment/Cellulose, studies in St. Maurice river, Québec). With such correlations, biochemical indicators could make good early warning signals, or "biomarkers" in the current vernacular [1]. The topic is discussed in meaningful fashion by Dixon et al. (1985).

CONCLUSIONS

Basic findings from both Scandinavia and North America retain their general validity. Scandinavian conclusions stand, that in general, high-chlorination BKME is more toxic than unbleached KME.

The absolute concentrations found harmful to aquatic organisms in the Baltic are not necessarily the concentrations that are applicable elsewhere.

Differences of one or two orders of magnitude in NOECs are still not clearly or completely explained. Recent work in both locations, discussed in other papers of this session, is resulting in greater similarity of findings.

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[1] I will venture a definition of biomarker in the context of aquatic toxicology, derived from SPOPEAE (1989). Biomarkers are biological manifestations of stress, usually at the biochemical, physiological, or morphological level, which are useful for estimating biological effects or exposure to chemical contaminants.

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APPENDIX. ADDITIONAL INFORMATION ON BALTIC PREDATORS AND BIOACCUMULATION.

Since 1960 there have been severe reproductive problems in Baltic marine mammals and predatory birds. Olsson (1987) says: "We know today that the reproductive rate of the Baltic eagles started to decrease in the late 1950s. We have good reason to believe that the reproduction rate of the Baltic seals decreased during the same time. ... Twenty years later, both the Baltic sea eagle and the Baltic seals are close to extinction. The severe situation for the seals was not discovered until the late 1960s, and that the reproductive rate was decreased was not discovered until 1974. In fact, it was not discovered until about 50% of the adult female seals were sterile." Many seals still swimming around "are non-reproductive and biologically dead."

The six mammalian species that inhabit the Baltic and feed on marine fauna are mink, otter, ringed seal, harbour seal, grey seal and common porpoise. "For all of these species the population numbers have decreased during the last 30 years, and for all except mink the changes have been dramatic" (Olsson 1987).

There is certainly a correlation with environmental pollution. For example, Norwegian otters, distant from the Baltic, averaged ≈ 17 mg PCB/kg in fat while southern Swedish otters had ≈ 180 mg/kg. Ringed seals from the Gulf of Finland (i.e. Baltic) had mercury content of ≈ 48 μg/g dry weight in the liver, PCB 300 μg/g dry, and ΣDDT 300 μg/g dry in the blubber (Perttilä et al. 1986). In the 1970s, only seals from the Netherlands had higher levels of PCB and only sea lions from California had higher values for ΣDDT (Holden 1978).

A low reproductive rate of ringed seals in northern Bothnia Bay (Baltic) was reported in 1979 -- only 25% pregnancy rates compared to 65-90% in the Soviet Arctic. PCB accumulations were thought to be responsible for abortions. Abnormalities in the uteri of adult females (occlusions or stenoses in one or both uterine horns) increased from 34% of the females in 1974-75 to an astonishing level of 59% in 1977-79 (Helle 1981). Similar abnormalities were found in females of grey and harbour seals, from all parts of the Baltic.

The "disease complex" reported for Baltic seals indicates an abnormally increased adrenalcortex activity that may secondarily lead to disturbances in the metabolism and the immune defence. Damages in the skeleton are also frequently found, particularly in the jaw region. All lesions are found at "an alarmingly high frequency" (Jansson et al. 1987).

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A CRITICAL REVIEW OF SCANDINAVIAN STUDIES ON THE AQUATIC IMPACTS OF PULP AND PAPER EFFLUENTS

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ABSTRACT:

Scandinavian environmental scientists have compiled a large body of research on the aquatic impacts of pulp and paper effluents. This work encompasses biological levels of organization ranging from community to subcellular responses. At the community and the population organizational levels, organic enrichment is the primary cause of ecosystem disruption. The present focus of concern, based upon sublethal measurements, is the conclusion that chlorinated organics in bleachery effluents are detrimental to fish populations. These conclusions were drawn by comparing a highly contaminated area suffering eutrophication against a single remote control area. Analysis of these data shows that many statistically significant differences between the mill and the control sites are within the natural variability for these measurements. The remaining data suggest reproductive and liver toxicity as endpoints for further study, but do not conclusively link the observations with organochlorine contaminants. In order to clarify these findings, future investigations should include (1) redundant chemical and biological measurements to assess specific endpoints and (2) an integrated assessment of chemical exposure and habitat alteration.

Introduction

The pulp and paper industry is the largest industrial wastewater source in Finland and in Sweden. The recipient waters of pulp and paper wastewaters are primarily freshwater lakes in Finland and the brackish inlets of the Baltic in Sweden (Bonsor et al. 1988; Fallenius 1987). The Finnish lake systems are characteristically shallow and sensitive to eutrophication, and the Baltic Sea is a unique ecosystem which is under considerable stress from anthropogenic pollution (Larsson et al. 1985a; Rapport 1989; Voipio 1981). Therefore, considerable environmental research effort has been devoted to pulp and paper discharges in these systems.

Historically, environmental impacts from pulp and paper discharges have been attributed to (1) eutrophication and enrichment and (2) deposition of fiber and suspended solids. In Finnish lake systems widespread impacts were observed with wholesale changes in the structure and function at the community and population levels (Eloranta 1970 and 1980; Tuunainen et al. 1972; Kansanen 1981; Kansanen and Aho 1981). In Swedish waters extensive impacts on benthic populations (Landner et al. 1977) and fish populations (Hansson 1987; Neuman 1986) were observed. As a result both countries have implemented regulations and have successfully reduced the volume of conventional pollutants (Bonsor et al. 1988).

The focus of environmental concern in Scandinavia has now turned to organochlorines now measured as Adsorbable Organic Halogens (AOX). The impetus was a series of biochemical and physiological measurements on fish exposed to bleached kraft mill effluents in both laboratory and field situations (Andersson et al. 1987; Andersson et al. 1988; Härdig et al. 1988). These investigators implicated organochlorines in bleachery effluents as the primary cause of the observed effects (Andersson et al. 1988).

Definitive conclusions that certain chemicals cause biological impacts requires the integration of several sources of information. These sources include: (1) chemical analyses, (2) chronic toxicity tests, (3) field measurements of community and population responses, and (4) sublethal responses at the organismal and suborganismal levels under both controlled and field conditions. Chemical analyses of effluent, receiving water, and sediment clarify which contaminants are produced by the mill, the chemical gradient of contamination, and the environmental transprot of contaminants. Chemical analyses of the biota describe both potential biotransformation reactions and the bioavailability of contaminants via bioconcentration and biomagnification. Chronic toxicity tests at several taxonomic levels approximates the toxicity of the effluent using either sensitive life stages or full life cycles. When the actual receiving waters are used as the effluent diluent, the contribution or the mitigation of toxicity by these waters is tested. Relevant impacts on structure, function, and reproduction can be measured at the community and the population levels. Effects on individual health can be measured at the organismal level, and acute, adaptive, and chronic responses to effluents may be measured at the suborganismal level. However, all measurements must take into account variability within a population to habitat, season, species and sexual cycle. Chronic exposures under controlled conditions provide an especially useful means to test for cause and effect. However, chronic exposure should include an intact food chain in order to reflect possible biotransformation and biomagnification. Otherwise, certain impacts may be observed to their full extent only in natural receiving waters.

The purpose of this review of Scandinavian studies on pulping effluents is then to ask the following questions:

1. Is the data sufficient to state that effluent organochlorines are causative agents for environmental impacts?

2. If sufficient, which organochlorines of several hundred such compounds in bleachery effluents may be responsible?

3. If insufficient, is there an investigative strategy which will resolve these questions?

Chemical Analyses

No Scandinavian study has analyzed all necessary chemical compartments: effluent, receiving waters, sediments, and biota. The basic conventional effluent parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS) are often lacking, and specific chemical analyses may be limited or even absent. As a result, exposure of organisms cannot be adequately assessed. The two most recent studies were at Lake Saimaa in Finland and Norrsundet on Sweden's Baltic coast (Oikari et al. 1980; Oikari et al. 1985; Oikari and Kunnamo-Ojala 1987; Xie et al. 1986). The Lake Saimaa effluent concentrations in the receiving waters were reported to be diluted 15 to 30 fold (Oikari et al. 1985) and the Norrsundet dilutions were reported to be 140 to 1000X fold (Andersson et al. 1988). At Lake Saimaa the analyses were primarily for chlorophenolics and resin acids in the effluent, receiving water, and biota. However, sodium, color, and oxygen levels were also analyzed at the biological sampling sites. At Norrsundet only chloroform and chlorophenolics in the effluent and receiving waters were analyzed. No analyses were performed on the remote Forsmark control site, the local Kusön control site, or the unbleached mill at Sandarne. These measurements were made prior to major mill modifications and the field work. The chemical sampling sites were at different locations than the biological stations, so that the actual conditions during the field study can only be approximated. In addition, the dilution estimates used for the Norrsundet field studies may be inaccurate. The estimates are based on chloroform concentrations from samples at five meter depths, while the fish were sampled nearby at two to three meter depths. Other data show that the mill effluents were layered on the surface (Xie et al. 1986), with concentrations at five meters from 4 to 20 fold lower than the biological sampling depths. Finally, the modifed Norrsundet mill had extensive start up problems during the field study. Black liquor discharges caused COD limits to be exceeded sometimes by over 100% for up to eighteen months (Södergren 1988).

Bioaccumulation is the best measurement of actual exposure, given variations in bioavailability and time of exposure. At Lake Saimaa Oikari and coworkers have studied bioaccumulation in caged rainbow trout, caged native plankton-feeding whitefish (*Coregonus muksum*) and native feral fish. Chlorophenolics and resin acids were found to be bioaccumulated and excreted in the bile as glucuronide conjugates (Oikari and Holmbom 1986; Oikari et al. 1984a; Oikari and Änäs 1985). The bioconcentration factors in rainbow trout plasma ranged from 80 to 800 for resin acids and from 100 to over 1200 for chlorophenolics. The chlorophenolics showed an apparent relationship between bioconcentration and the degree of chlorine substitution of the phenolic ring (Oikari et al. 1985; Oikari and Kunnamo-Ojala 1987).

At Norrsundet the bioaccumulation of 3,4,5 and 4,5,6-trichloroguaiacols in bile, dioxins and furans in fillet, and EOX levels in lipid have been measured in feral perch (Perca fluviatilis) (Södergren 1987 and 1988). Compared to Lake Saimaa, the 4,5,6 isomer bioconcentration is lower at Norrsundet, supporting greater exposure of perch at the Finnish site. The 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) levels in fillet ranged from 2.6 to 19 parts per trillion, implicating the Norrsundet mill as a source for dioxin (Södergren 1988). Additional compounds such as resin acids were not analyzed. High background levels of EOX, 50-120 micrograms per gram of lipid, were found at an local unpolluted control site (Kusön), the unbleached Sandarne mill and even freshwater sites (Södergren 1987 and 1988). These data are consistent with recent findings that natural background levels are present in most surface and subsurface waters in Scandinavia (Asplund et al. 1989). EOX levels at stations over 5 kilometers from the discharge were at this background range throughout the study period. Within 5 kilometers of the discharge, EOX bioaccumulation rose near the discharge and decreased in the receiving area when the mill's chlorine use declined (see Table 1).

Table 1. Summary of EOX bioaccumulation, chlorine use and effluent characterization at the Norrsundet mill.

Year	EOX in Perch[a] (ppm in lipid) Distance from Outfall 1 km	3 km	4.5 km	Chlorine[b] Used (kg/ton)	BOD_7 (kg/ton)	COD (kg/ton)	TOCl (kg/ton)	TSS (kg/ton)
1982	—	—	—	88.2	31.1	127.7	7.5	5.4
1983	423	215	—	58.7	29.1	108.7	5.9	3.5
1984	330	295	151	37.4	20.1	69.8	3.8	2.4
1985	180	130	91	30.5	15.7	51.9	—	2.6
1988	—	—	—	20.1	14.3	52.7	1.7	1.5

[a] Data on EOX from Södergren (1987).

[b] Data on Norrsundet discharge parameters from Kautsky et al. (1988) and Neuman and Karås (1988).

History of Norrsundet mill modification during 1980's: 1982 and before - Norrsundet had both bleached and unbleached mills. The bleached mill was without oxygen delignification and used molecular chlorine. 1983 - Mill conversion begun and oxygen delignification system installed. Mill authorized for COD discharges of 40 kg/ton, but black liquor discharges cause limits to be exceeded well into 1984.

1987 - Reduction in molecular chlorine use with chlorine dioxide substitution in the bleach plant.

No chronic toxicity tests analogous to the fathead minnow or Ceriodaphnia protocols have been performed in parallel with field or laboratory studies of pulping effluents in Scandinavia. Acute toxicity tests, based upon 96 hour LC50 procedures, are also rare. In the case of long term exposures to bleached kraft effluents the acute toxicities of these untreated effluents were 96 hour LC50's of 8% (Andersson et al. 1987; Härdig et al. 1988). At Lake Saimaa caged rainbow trout acute toxicities were observed in 7 out of 10 fish 3 kilometers from the mill (Lindström-Seppä and Oikari 1990).

Field Measurements in Waters Receiving Pulping Effluents

Disruption at the community and the population levels is accepted as a relevant environmental impact. In Scandinavia Landner et al. (1977) surveyed the benthic communities at fifteen Swedish pulp and paper sites, including the Norrsundet mill. Zones near all types of mills including bleached and unbleached were often devoid of benthos due to anoxic deposits of fiber mats with subsequent transition into enrichment zones from the discharge of untreated effluents. More recent surveys indicate damage still exists in these areas with no indication that benthic species respond differently to bleached or unbleached effluents (Cederwall and Blomqvist 1987). Finnish lakes had also experienced severe disruption from pulping discharges (Tuunainen et al. 1972; Kloranta 1970 and 1980; Kansanen 1981).

The most recent community level work is that of Kautsky et al. (1988) at the Norrsundet mill. Severe changes in the structure and the function of the community were observed. A fiber mat was present in the innermost area which was nearly devoid of benthic life. Dense masses of green algae, Vaucheria species and Cladophora aegagrophila, were present at the surface layers at the inner station, and 40% of the faunal biomass at the inner station was the pollution tolerant and bacterial feeding hydrozoan Cordylophora caspia. A succession of plant communities occurred with distance from the discharge as plant biomass increased and colonization occurred at greater depths. The faunal diversity and biomass followed similar succession trends, and as shown in Figures 1 and 2 for taxa and biomass, respectively. Kautsky et al. (1988) concluded that eutrophication was the cause for the disruptions and noted the similarity between their observations and those of Pearson (1980) and Pearson and Rosenberg (1978). No community data are available for the Lake Saimaa study or the Sandarne and Forsmark sites in the Norrsundet study.

Fish Population Studies

The fish distributions at Swedish mills have been reviewed by Hansson (1987) and Neuman (1986). Common observations are increases in cyprinids, roach and ruffe, at untreated pulp and paper sites in the Gulf of Bothnia while perch populations are reduced near the discharges then increase with distance from the mill. Hansson (1987) notes the similarity in pattern regardless of the process chemistry. Neuman (1986) has reviewed the existing fish distribution surveys in Sweden and concludes that (1) eutrophication was the principle cause for the alterations and that (2) no unique or increased level of effects could be observed near organochlorine discharges.

The fish populations at Norrsundet have been intensively studied. The target fish species were roach (Rutilis rutilis) and perch (Perca

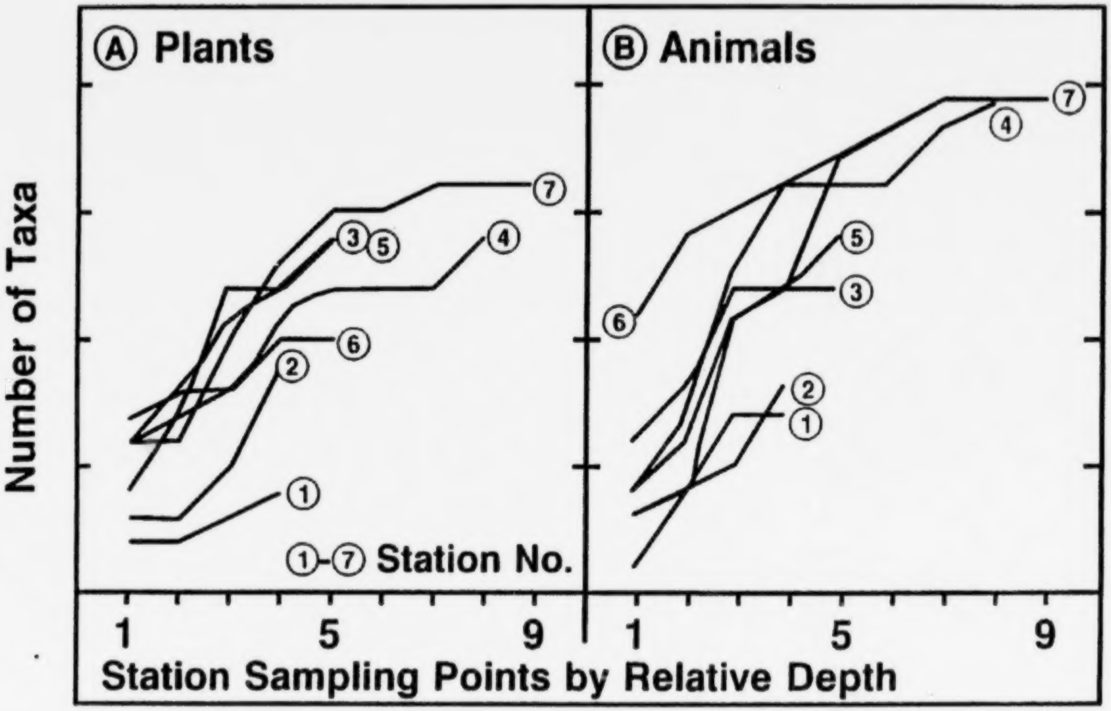

Figure 1. Number of flora and faunal taxa at Norrsundet sampling stations.

Legend

The total number of flora (A) and faunal (B) taxa found at the sampling stations are plotted by relative depth at each station. The data are from Kautsky et al. (1988). With permission.

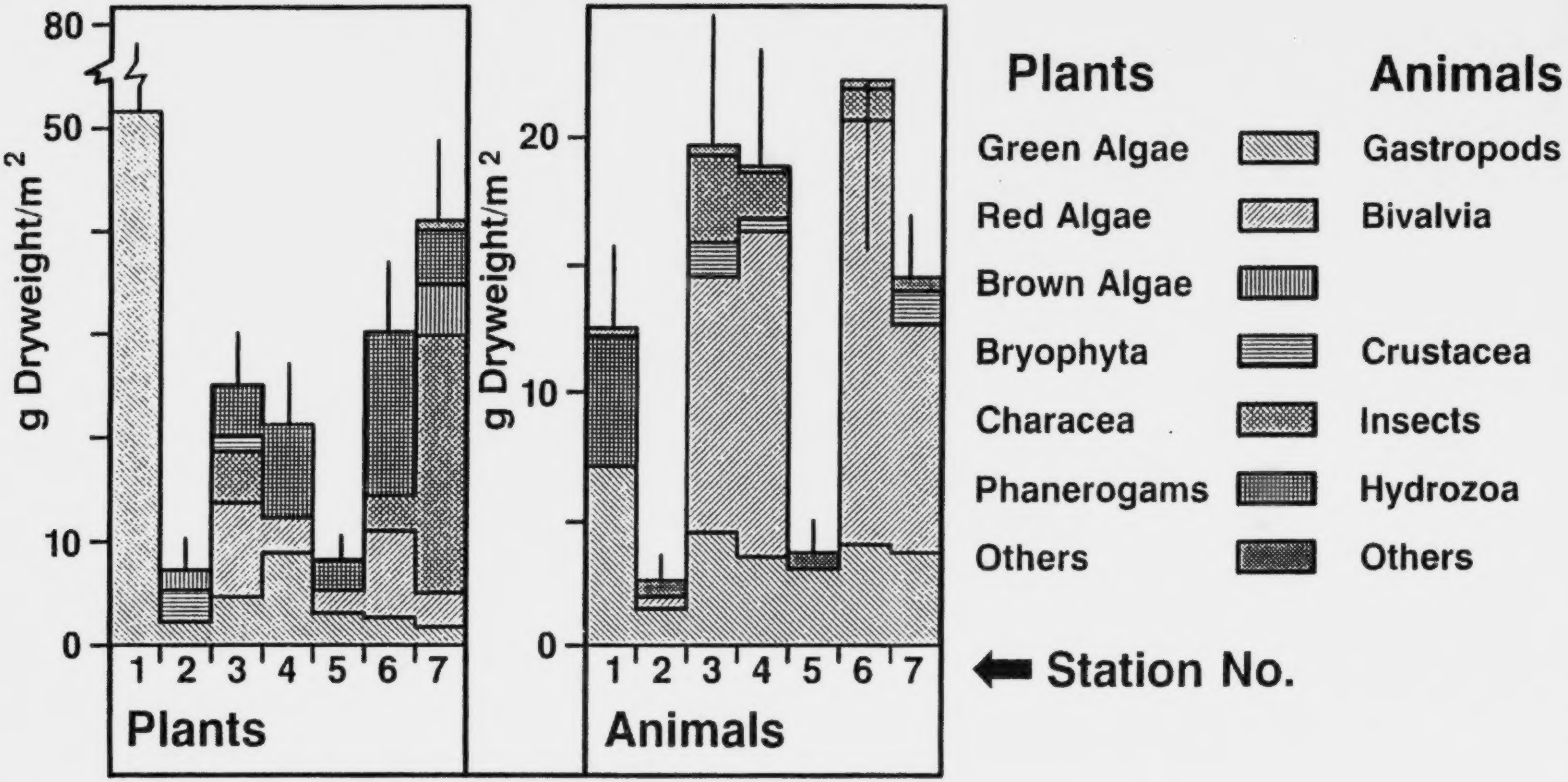

Figure 2. Biomass of flora and fauna at Norrsundet sampling stations.

Legend

The average biomass of major plant and animal taxa are summarized for each sampling station. The standard deviation of the total is shown by the height of the vertical line above the stacked bar. The major groups are shaded with the legend for plant and animal groups on the right. The data are from Kautsky et al. (1988). With permission.

fluviatilis), which are common throughout the coastal regions of the Baltic. The distribution of species occured as described by Hansson (1987) and Neuman (1986) at both Norrsundet (bleached) and Sandarne (unbleached) (Neuman and Karås 1988). The shallow zone nearest the Norrsundet discharge was almost devoid of fish. Cyprinid populations, roach and ruffe (Gymnocephalus cernua), were high in an intermediate zone while perch populations were limited. In a more distant zone perch populations increased to become the predominate fish species. The unbleached Sandarne mill discharges less than one third of the COD and the BOD as Norrsundet (Södergren 1987). Consistent with the smaller discharge and more open site, no zone was devoid of fish while the respective roach and perch zones were closer to the discharge. The authors concluded that eutrophication was the principle cause of the altered distribution with possible chemical toxicity in the innermost Norrsundet zone where fish were absent (Neuman and Karås 1988; Neuman and Sandström 1988).

Perch reproduction and recruitment have been examined at Norrsundet. Sandström et al. (1988) measured the readiness of perch to spawn by gonad classification. At Kusön and Sandarne 75% of the female perch were judged to be in the spawning cycle. Only 45-60% of the females were judged ready to spawn 2 kilometers from the discharge, 65-70% of the females were ready to spawn at 3 kilometers, and readiness to spawn was not affected in the remaining area. Gonad somatic index (GSI) values were measured and found to be lowest nearest the discharge and rising to control levels with distance. In subsequent studies, perch have been found to successfully spawn in the shallows at the mill discharge. However, several developmental trends in the discharge area indicated stressful conditions: embryo abnormalities were slightly higher (10%) than controls (1%) and embryo growth was decreased. After hatching the larval mortality of perch in the exposed area approached 100%. The authors concluded that acute toxicity and parental weakness, possibly from exposure to toxic chemicals, were the causative factors for the mortality (Karås et al. 1991). This is in contrast to successful reproduction by the more highly exposed roach populations in this area. Otherss have suggested that effects of color and turbidity may occur on larval feeding behavior citing the work of Dabrowski and Hinshaw (Neuman and Karås 1988). Dabrowski (1982) has shown that roach larvae feed under low light conditions while perch require higher light levels for successful feeding. Hinshaw (1985) observed that perch larval survival fell from 44% in high light conditions to only 1% in low light. Supporting the visual hypothesis, Karås et al. (1991) observe that only large zooplankton are in the stomach of surviving larvae even when the normal diet of smaller prey are abundant in the area.

Growth in both perch and roach increased near the mill discharge in both juvenile and adult life stages (Sandström et al. 1988; Sandström 1987). At the inner site growth rates are high when compared to other Baltic observations for perch (Neuman 1976; Hansson 1985; Hansson and Westin 1985). The higher growth rate in perch at Norrsundet may be due to several factors: (1) low population densities at the inner stations, as an inverse relationship exists between growth and population density (LeCren et al. 1958), (2) eutrophication, as growth accelerations occur in both perch and roach under eutrophic conditions (Hartmann, 1978 and 1982), and (3) reduced reproductive effort at the innermost station, but this would not explain increased juvenile growth.

Increased perch mortality rates based on age profiles have been observed at the inner Norrsundet sites (53%) versus the local Kusön control site (44%) (Sandström and Thoresson 1988). The control data agree closely with the 41% rate observed in long term experiments on perch elsewhere (LeCren et al. 1977). Mortality rates were supported by following perch scarred by fin erosion, and migration of these fish to outer stations was not observed. The mortality rate in the scarred population was 59% (Sandström and Thoresson 1988). The cause of the mortality remains in question although fin erosion occured during the period of high black liquor discharge. Seasonal data are also needed as Norrsundet's ice cover of nearly five months would increase the local stress (Neuman and Karås 1988).

Laboratory Exposures to Pulping Effluents

Long term exposures have been conducted with three spine stickleback (Gasterosteus aculeatus), rainbow trout (Salmo gairdneri), and fourhorn sculpin (Myoxocephalus quadricornis) in mesocosms and the laboratory. The exposures include several effluent types, and measurements range from early life stage growth and spinal morphology to histopathology and biochemical/physiological tests.

Three spine sticklebacks were exposed for nine months in the laboratory to untreated bleached kraft mill effluent (BKME) from the Husum mill in Sweden (Andersson et al. 1987; Härdig et al. 1988). The results of biochemical and physiological measurements are compared against field samples of perch for similar measurements in Table 2. Except for the induction of mixed function oxidase (MFO) activity against an 7-ethoxyresorufin deethylase (EROD) substrate and chloride ion, the field and the laboratory samples did not respond in a similar manner for these biochemical changes.

Sublethal measurements were made from mesocosm exposures on rainbow trout (Lehtinen 1990) and three spine stickleback larvae and fry (Lehtinen et al. 1990) lasting up to five months. Several effluents were utilized in these experiments which included molecular chlorine bleaching, high chlorine dioxide substitution, and oxygen delignification processes. These effluents were both treated and untreated. The results showed that untreated effluents from mills using high levels of molecular chlorine had the strongest negative effect on the survival and growth of larvae, histopathological changes in liver such as pynocytic nuclei and vacuolization, and biochemical measurements such as EROD induction. Modified processes with oxygen delignification and effluent treatment partially alleviated the effects. The most effective process modification was the substitution of chlorine dioxide for molecular chlorine which was equivalent to controls (Lehtinen 1990; Lehtinen et al. 1990). The authors noted that the sublethal measurements varied within control sets to encompass numerous statistical differences between exposed and control pair groups. This led to the interpretation, that since these differences fell within natural variability range, the effects were adaptive and not a severe disruption (Lehtinen et al. 1990).

Fourhorn sculpin and bleak (Alburnus alburnus) were exposed to several pulping effluents, including unbleached, untreated BKME, and treated BKME, and the impact on the development of spinal deformities was measured (Bengtsson et al. 1988). The deformities observed in the laboratory were less pronounced than in feral samples, and no significant differences were observed between the impacts of unbleached and bleached effluents. The effluent most like the control was a molecular chlorine process which had been treated in aerated lagoons (Bengtsson et al. 1988), and the author concluded that the presence of chlorinated materials did not appear to be cause the deformities (Bengtsson 1988).

Chronic laboratory exposures have also been performed in Finland. Tana (1988) exposed rainbow trout to both chlorinated phenols and resin acids for 80 days. Several parameters initially changed from control values at ten and twenty days, but then returned to control levels. No substantive differences in the response of sublethal measurements of blood glucose and lactate, liver glycogen, leucocrit, hematocrit, or mean cell hemoglobin concentration were observed between chlorophenolics and resin acids. There were different trends in UDPGT responses (Tana 1988). These experiments identify two concerns for future studies. Firstly, acute exposures may induce sublethal responses which decrease with time as adaptation occurs, suggesting that (1) field studies should focus on species with a limited home range so that exposure is relatively constant and errors due to migration are controlled, and (2) caging experiments should have adequate time to distinguish between acute and adaptive responses. Secondly, many sublethal measurements may react similarly with a number of chemical contaminants in complex effluents.

There are experiments which point to specific contaminants. Laboratory exposures to resin acids caused significant changes in blood bilirubin levels and UDPGT with some vacuolization of liver tissue (Mattsoff and Oikari 1987; Oikari et al. 1984b). Differences were then observed between whole bleached kraft mill effluent (BKME) and purified fractions. When fish were exposed to whole mill BKME, EROD activity was induced several fold (Oikari and Lindström-Seppa 1990). With purified resin acids and chlorophenolics there was only a slight stimulation of EROD activity (Oikari et al. 1988). In subsequent experiments, strong induction was seen with 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), and the authors propose that the intense EROD induction from BKME is due to TCDD and other highly chlorinated planar hydrocarbons (Oikari and Lindström-Seppä 1990). These findings are consistent with comparisons of the effects of coplanar and non-coplanar polychlorinated biphenyls (PCB's) (Gooch et al. 1989) and 2,3,7,8-tetrachlorodibenzo-p-furan (TCDF) (Hahn et al. 1989) on EROD activity induction. However, EROD induction also occurs with polyaromatic hydrocarbons from combustion and petroluem products. Both may occur where boiler ash is introduced into wastewater streams and petroleum based process defoamers are used.

Physiological and Biochemical Measurements in Field Exposures

Vertebral and spinal deformities in the Baltic fourhorn sculpin (*Myoxocephalus quadricornis*) are the most intensively studied physiological endpoint of pulping effluents. In the field these deformities are widespread with background rates of about 20%. At the Husum bleached kraft mill, the most intensively studied site, deformity rates range from 10% to 60% depending upon the distance from the mill and the time of sampling. However, no contaminant analyses of the water column, sediment, or biota were performed to quantify exposure. At the Köpmanholmen and Sundsvall bleached kraft mills deformity rates ranged from 30-60%. Conversely, at the Jakobstad and the case study Norrsundet bleached kraft mills deformity rates were either below or near background at 9 and 16%, respectively. The authors note that vertebral deformities increase in habitats at greater depth and lower water temperature in addition to proximity to mills (Bengtsson 1987).

Tana and Nikunen (1986) analyzed a series of parameters in short ten day exposures of caged rainbow trout: hematocrit, hemoglobin concentration, serum ion concentrations, blood levels of glucose and lactate, and serum total protein. Changes were seen in several parameters with distance from the discharge which were statistically significant. In extending the studies over four seasons, variations in control values with season were equal to or greater than changes between controls and exposed individuals. The authors noted that the greatest deviations occurred during March sampling when oxygen saturations were lowest after an extended winter ice cover. The authors suggested that to obtain more meaningful interpretation, future experiments should be conducted with additional stressors such as exercise (Tana and Nikunen 1986).

Oikari and coworkers caged rainbow trout in a series of experiments of varying length and assessed several biochemical and physiological responses: liver uridine diphosphate glucuronosyl transferase (UDPGT), blood hemoglobin, plasma protein concentration, plasma ion concentrations, and other measurements. Episodic increases and decreases were seen in UDPGT activity, and serum sodium concentrations were elevated in parallel with the water column sodium concentrations from the effluent sodium discharges. Although several parameters were statistically different from controls on one or more occasions, there was no correlation with either the chemical gradient or bioaccumulation (Oikari et al. 1985).

Rainbow trout and two species of whitefish were caged for 21 days in the latest studies at Lake Saimaa. The whitefish as plankton feeders can be caged for extended periods and maintain the normal food chain. In addition, feral fish were captured near the same stations. UDPGT activity and osmoregulation were not effected by effluent exposure in caged whitefish, while UDPGT rose slightly in rainbow trout (Lindström-Seppä and Oikari 1989 and 1990a,b). Cortisol concentrations in exposed rainbow trout were similar to control levels (Lindström-Seppä and Oikari 1990a). Both whitefish and rainbow trout showed significant elevations of liver mixed function oxidase (MFO) activities: arylhydrocarbon hydroxylase (AHH) and EROD (Lindström-Seppä and Oikari 1989 and 1990a). EROD induction was also observed in feral perch, roach, and bream near the mill and species differences in the degree of MFO induction (bream > perch = roach). At several unpolluted sites a wide range of background MFO levels were found in feral fish, and the authors noted the importance of assessing several sites for background range and representativeness. Glutathione levels were also increased in all feral species near the mill discharge which the authors suggested was an adaptive response (Lindström-Seppä and Oikari 1990b).

Swedish biochemical and physiological measurements on perch (Perca fluviatilis) includes on a large body of work at metal polluted and normal sites (Larsson et al. 1984; Larsson et al. 1985b; Sjöbeck et al. 1984). These studies provide valuable background data to assess natural variability, and one study was designed to quantify background noise variation (Larsson et al. 1985b). These assays were applied to feral perch at Norrsundet (Södergren 1987; Andersson et al. 1988; Södergren et al. 1988) and to fourhorn sculpin (Myoxocephalus quadricornis) in laboratory exposures (Andersson et al. 1987; Härdig et al. 1988). Unfortunately, the Norrsundet results were only analyzed by statistical differences to the remote Forsmark site, and the extensive variability data base was not utilized for interpretation.

At Norrsundet over twenty biochemical and physiological measurements were performed on the feral perch collected during four different sampling periods. These data are assembled in Table 2 (1) to assess the consistency of the observations over time, (2) to include data on natural variability, and (3) to compare the field results to the laboratory exposures. Several measurements do not show any statistical difference, e.g., blood sodium and white blood cell number, or show inconsistent patterns, e.g., blood glucose and thrombocyte numbers. Twelve measurements from the inner stations are statistically different from Forsmark in three out of four sampling periods. About half of these differences fall within normal observed range of the species, e.g. blood chloride and mean cell hemoglobin. Some measurements do not appear to indicate deterimental effects, but support better growth and nutrition in the Norrsundet perch, e.g., higher blood glucose and higher liver glycogen and muscle glycogen. In this respect, the hematocrit values are noteworthy: the values from the exposed perch are

Table 2. Summary of Biochemical and physiological measurements at Norrsundet, control sites, and laboratory studies.

	FIELD[a]				Kusön	LABORATORY[b]	
Sample Date:	5/84	8/84	10/84	9/85	9/85	Pine	Birch
EOX in Lipid (ppm)	nd	300	nd	170	nd	320	nd
Assay							
Liver somatic index	+	+	+	+	NS	+	NS
Gonad somatic index	nd	-	-	-	NS	nd	nd
Blood sodium	NS	NS	NS	NS	$-^c$	NS^d	NS
Blood chloride	$-^c$	$-^c$	$-^c$	$-^c$	$-^c$	$-^d$	NS
Blood potassium	$+^c$	NS	NS	NS	-	NS^d	-
Blood calcium	NS	$-^c$	$-^c$	$+^c$	NS	NS^d	NS
Blood magnesium	$+^c$	$-^c$	$-^c$	$+^c$	-	NS^d	NS
Blood glucose	$-^c$	NS	NS	$+^c$	$+^c$	NS^d	nd
Blood lactose	+	NS	+	$+^c$	NS	NS^d	nd
Liver glycogen	NS	+	$+^c$	NS	-	nd	nd
Blood bilirubin	+	+	NS	+	NS	nd	nd
UDP-glucuronotransferase	+	NS	+	NS	NS	NS	NS
Muscle glycogen	NS	$+^c$	+	NS	NS	+	NS
Hematocrit	$+^c$	+	$+^c$	$+^c$	NS	NS	-
Hemoglobin	$+^c$	NS	NS	$+^c$	$+^c$	NS	-
RBC number	+	+	+	+	$+^c$	-	NS
Mean cell hemoglobin	$-^c$	$-^c$	$-^c$	-	NS	NS	-
Mean cell volume	-	-	-	NS	NS	+	NS
Met-hemoglobin	+	nd	+	+	NS	-	NS
WBC number	NS	NS	NS	-	$-^c$	NS	NS
Lymphocyte number	NS	$-^c$	$-^c$	-	$-^c$	NS	NS
Thrombocyte number	NS	NS	+	$-^c$	NS	NS	NS
Granulocyte number	+	NS	NS	NS	NS	NS	NS
EROD	+	+	+	+	NS	+	NS
Ascorbic acid	+	+	+	+	NS	nd	nd
ALA-D	NS	+	-	+	$+^c$	nd	nd

EOX data (Södergren 1987), field data (Andersson et al. 1988), laboratory data (Andersson et al. 1987; Härdig et al. 1988) control background data from (Larrson et al. 1984; Larsson et al. 1985b; Sjöbeck et al. 1984).

nd: Not done, NS: Not significant, +: Significantly higher than control ($p<0.01$) by Duncan's new multiple-range test, -: Significantly lower than control by same statistical measure.

[a] Innermost Norrsundet site at two kilometers.

[b] Husum mill BKME, pine and birch bleach line effluents diluted to 0.6%.

[c]: Signficantly lower than control, but still within normal range.

[d]: Data available only for lower dose (0.12%) of pine effluent.

near normal values while controls are lower (see Figure 3). In fact, the hematocrit values of the Forsmark controls have previously been judged to represent slightly anemic conditions (Sjöbeck et al. 1984).

The remaining measurements indicate that there are multiple toxicants in the Norrsundet receiving waters. The increased methemoglobin values reflect reducing agents such as nitrite in eutrophic waters. An increase in bilirubin levels, smaller erythrocytes, and lower cell hemoglobin contents were all effects from chonic resin acid exposures (Matsoff and Oikari 1985; Oikari et al. 1988). Increased liver somatic index and EROD activity induction raise concerns for hepatotoxic effects. However, high ascorbic acid values did not indicate that chemical pools necessary for detoxification were depleted. In contrast to the Norrsundet liver somatic index results, exposures with up to 5% BKME did not cause liver enlargement in rainbow trout (NCASI 1989; Oikari and Niittylä 1985). GSI values raise questions about reproduction, but similar findings to Norrsundet have been observed at unbleached mills at Piteä where loadings and turnover restrictions closer to Norrsundet's (see Table 3) (Bergelin 1987; Bergelin et al. 1986). Samples for biochemical and physiological analyses were collected at Sandarne and indicated a lower degree of response (Larsson et al. 1988), but these data have not been published. Furthermore, none of the parameters appears to show a dose response to EOX levels, which fall dramatically during the study (see Tables 1 and 2).

Discussion

Scandinavian data demonstrate the difficulties in working with complex effluents and defining which contaminants may be responsible for

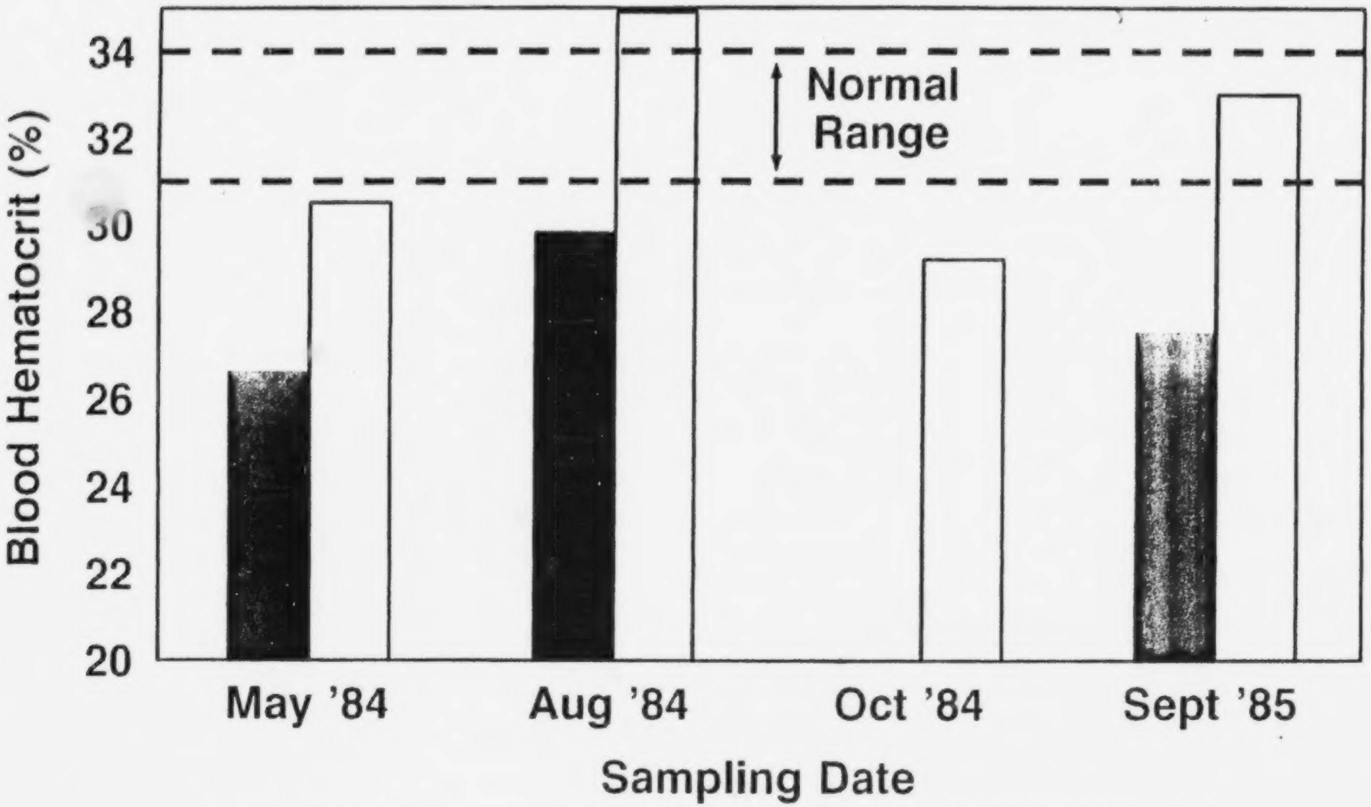

Figure 3. Hematocrit values of Norrsundet and Forsmark perch compared to background measurement variability.

Legend

Hematocrit values for female perch at each of four sampling periods at Norrsundet and Forsmark (Andersson et al. 1988) are compared to background hematocrit measurements in female perch in unpolluted Swedish waters (Larsson et al. 1984; Sjöbeck et al. 1984).

Table 3.

Growth and reproductive effects in perch near Piteä unbleached kraft mills in the Gulf of Bothnia

	Reference Site	At Unbleached Discharges	
		Mill 1	Mill 2
Length (5 yr old males - cm)[a]	162	180	174
Length (5 yr old females - cm)	171	189	174
GSI (male)[a]	5.98%	3.63%	2.41%
GSI (female)[a]	1.90%	0.96%	1.23%
Sexually Mature (female)	59%	combined 43%	

Data are from Bergelin et al. (1986).

[a] Differences in male length and GSI values for both sexes were statistically signficiant ($p < 0.05$) by student's t-test.

environmental effects. When chemical and chronic toxicity data are absent, it is difficult to support cause and effect conclusions. In the Norrsundet case AOX and EOX measurements were frustrated by high background levels, and these measurements are no longer recommended for attempting to trace organochlorines from bleacheries (Södergren 1988). Toxicity data would clarify the caging studies and population distributions which indicated acute toxicity may be present in some receiving situations. Community and population data show that organic enrichment can confound the field situation, and make interpretation of other measurements difficult. Scandinavian data reveal that biochemical and physiological responses have both considerable natural variability and also acute and adaptive stages. Both conditions make interpretation of sbulethal data difficult without extensive background data bases. Efforts to reproduce field effects in controlled situations using either whole effluents or purified fractions, such as resin acids and chlorophenolics, have not been highly successful. Comparisons of effluent types in chronic exposures do indicate that responses decrease with (1) the biological treatment of effluents and (2) the substitution of chlorine dioxide for molecular chlorine.

The role of changes in habitat on sublethal measurements needs further investigation. The Norrsundet situation specifically show the confounding effects that a highly polluted environment and multiple contaminants may have on sublethal measurments. The field data show a high degree of eutrophication and enrichment altering the entire community including the fish populations. Although community data are not available for Sandarne, similar trends for fish population distributions were seen with the degree of symptoms consistent with the smaller discharge load at Sandarne. Without data to assess the impact of these habitat changes on the biochemical and physiological measurements, conclusions that organochlorines cause these effects are not justified.

The Scandinavian results show that careful control site selection and habitat characterization are necessary for proper interpretation. The Forsmark site is a good example as community and fish populations were not characterized. In addition, the growth of Forsmark perch is poor, and biochemical measurements of nutrition are not robust. Comparing biochemical and physiological data from Forsmark to the local Kusön control site, statistical differences exist for several biochemical and physiological measurements are evident in the September, 1985 sampling (see Table 2). Contamination of the Kusön site is unlikely as the current in this region flows from north to south, and plume analyses support this interpretation (Xie et al. 1986; Södergren 1987 and 1988; Kolset and Heiberg 1988). At Norrsundet age and growth differences exist with the Forsmark site in addition to organic enrichment changes in the habitat (Sandström 1987; Sandström et al. 1988). These variations were not incorporated into the interpretation of the Norrsundet data where only statistical differences were used (Södergren 1987; Andersson et al. 1988; Södergren et al. 1988). This suggests that statistical differences are not reliable to claim environmental impact. Validation of habitats, community structure, differences in growth rates and age composition, and background variability in sublethal measurements are all necessary to fully interpret results.

The ability of the biochemical and physiological measurements to support a conclusion that organochlorines (AOX) are the causative toxicants is limited. Several consistent differences which may be outside the background range have been induced by other toxicants. Particularly, there is no evidence for a dose response between organochlorines and the effects. The biochemical measures did not change with decreased organochlorine exposure: (1) decreasing molecular chlorine use at the mill or (2) lower EOX bioaccumulation (see Tables 1 and 2). In fact, the intensity of several sublethal measurements was strongest in September, 1985, when chlorine use and the EOX bioaccumulation were lowest, e.g. the lymphocyte counts. This suggest two possible explanations: the causative agents are not organochlorines or they are highly persistent organochlorines which remain in the environment after chlorine use decreased. As the contaminant analyses of biota indicate 2378-TCDD was present, the latter hypothesis needs further clarification.

Persistent compounds causing adverse effects after their production ceases seriously complicates future assessment programs. In particular, assessment of efforts to eliminate TCDD and TCDF production will be challenged. Clarification places increased emphasis on contaminant analyses of the effluent source and all environmental compartments in the future. If persistent compounds such as TCDD, TCDF, and other polychlorinated compounds do interfere with field monitoring, controlled conditions may be the only opportunity to assess new process effluents. For controlled exposures to reproduce field results and to test cause and effect, contaminants in the food chain should first be known by analyzing body burdens. Then these body burdens should be reproduced in the controlled exposure situations.

Of all measurements, the induction of EROD activity appears to be closest to being a test for exposure to organoclhorines. However, the induction by polyaromatic chemicals in combustion products and hydrocarbons still needs to be widely assessed. The overall lack of specificity for organochlorines forces a continued caution in interpreting results and a reliance on extensive chemical analyses for exposure assessment. A major unresolved question is the impact habitat differences may have on biochemical and physiological measurements in addition to specific chemicals.

This suggests a more structured use of most biochemical and physiological measurements. First, does the measurement contribute to the evaluation of a defined, relevant endpoint? Many previous measurements were based on non-specific parameters adapted from clinical tests of higher vertebrates. The long-term need is to evaluate the status of critical endpoints for organism health such as hepatic function and reproduction. Therefore, an initial tier comprised of a suite of measurements should be compiled to diagnose these critical endpoints. Second, given the natural variability of and limited experience with most tests, are single measurement methods adequate? The redundance of information from a suite of tests focusing on particular critial endpoint is needed. When combined with background data bases on variation and habitats, a suite of measurements provide less ambiguity than a single test. Third, where an endpoint dysfunction is clearly evident, further investigation to discover the responsible contaminant and the mechanism of action of that contaminant becomes possible. At this point a second tier of more detailed studies focusing on the dysfunction becomes possible with the objective of linking the effect with causative agents. The ultimate objective, when an agent is identified, is to establish effluent limits based upon both a no observed effect level and a margin of safety in the environment.

Conclusions

Scandinavian researchers have initiated efforts to establish cause and effect relationships between components in pulping effluents and biological effects at the sublethal level. These efforts have been hindered due to the chemical complexity of the effluent, the multiple impacts of pulping effluents ranging from eutrophication to chemical toxicity, and both the variable and non-specific nature of many sublethal measurements used. For these reasons, the data to date are not sufficient to link organochlorines in pulping effluents to particular biological effects. Future investigations should focus on meaningful endpoints most likely to be affected by organochlorines such as hepatotoxic and reproductive effects. A strategy integrating chemical, toxicological, community and population measurements with a suite of redundant sublethal measurements against these endpoints is recommended. The probability of success will be increased where data bases exist which provide both the background variation and the response in different habitats for the sublethal measurements used.

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EFFECTS OF A BIOLOGICALLY TREATED BLEACHED KRAFT MILL EFFLUENT ON RAINBOW TROUT (*Oncorhynchus mykiss*) PRODUCTION AND SUPPORTING FOOD WEB IN EXPERIMENTAL STREAMS

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The effects of an aeration stabilization basin biologically treated bleached pulp and paper mill effluent on coldwater streams were tested in large outdoor experimental streams (1). Two of the 110 m long streams received effluent additions and two other streams served as controls. Each stream received >1000 L/min water flow and consisted of an alternating pattern of riffles and pools. Annual effluent addition studies were conducted at effluent concentrations ranging from 0.5 to 2.0 mg/L of BOD_5 addition (1.3 to 5.1 % v/v) followed by a 3.5 year study at 0.5 mg/L BOD_5 addition.

Possible effluent effects were determined by sampling the resident rainbow trout (*Oncorhynchus mykiss*) population, and the supporting periphyton/macroinvertebrate food web. Each study was initiated with a new population of 1-2 g fish stocked at 3 g/m^2 Based on monthly sampling, the fish population was characterized in terms of cumulative production, histopathology, and reproductivity capacity. Concurrent effluent and streamwater analysis for chemical/physical parameters allowed in-stream biological responses to be interpreted relative to color, total suspended solids (TSS), chlorphenol, resin acid, and chlorinated resin acid concentrations.

The range of effluent addition studies included highest in-stream effluent concentrations of 3.5 mg/L TSS, 230 color units, 16.4 μg/L chlorophenols, 10 μg/L resin acids, and 2.5 μg/L chlorinated resin acids. The 3.5 year final study had corresponding concentrations of 1.0 mg/L TSS, 58-71 color units, 3.5 μg/L chlorophenols, 2.7 μg/L resin acids, and 0.5 μg/L chlorinated resin acids. Effluent addition was found to result in small reductions in dissolved oxygen and slight increases in temperature, ammonia nitrogen, and soluble reactive phosphorus. More marked were the effects of effluent color on underwater light transmittance.

Stream periphyton when measured as chlorophyll *a* indicated reductions at higher effluent concentrations for combined stream depths but enhancement at lower effluent concentrations or shallow stream depths. Periphyton when measured as biomass (ash-free dry weight) was greater at the highest effluent addition.

Macroinvertebrate communities were similar for all streams over the progression of effluent addition studies, including numbers and types of species present, species diversity, biomass, and density. Some shifts in species dominance occurred at the highest effluent addition level.

The progression of studies indicated a pattern of enhanced trout production, growth rate, and average weight with effluent addition. A corresponding pattern of reduced survival may be a normal density/growth relationship characteristic of a population of larger faster growing fish. Satisfactory trout production over the range of effluent concentrations indicated a lack of deleterious effects on trout either through direct effluent effects or indirectly through effects on the food web.

Trout histopathology for each of the studies indicated a lack of effluent related lesions or neoplasia. Effluent effects were contraindicated for blood hematocrit, leucocrit, or liver somatic index. The 3.5 year final study concluded with the in-stream spawning of one pair of control stream and two pair of effluent treatment stream fish. Resulting survival from egg deposition to hatching was 86 percent for control stream and 61 and 16 percent for treatment stream spawns. Although not a large sample size, these values fall within the range expected for survival to hatch in natural streams. A two-week follow-up laboratory growth study indicated effluent exposed fry achieved a greater mean weight gain and final mean weight than control fry.

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ADSORBABLE ORGANIC HALIDE (AOX) SAMPLING IN THE ATHABASCA AND WAPITI-SMOKY RIVERS FALL & WINTER 1989-90

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ABSTRACT

Adsorbable organic halide (AOX) has become an accepted measure of chlorinated organic material, and is used to monitor and regulate bleached kraft pulp mill effluents (BKME). Two surveys on the Wapiti-Smoky river system and one on the Athabasca River were carried out in 1989-90 to assess the presence and downstream persistence of AOX. Both of these river systems receive BKME.

AOX was found to be a convenient tracer of BKME: the effluents from the Weldwood of Canada mill at Hinton and the Procter and Gamble mill at Grande Prairie markedly increased AOX concentrations in the rivers. Downstream of the effluents AOX declined in concentration, primarily due to dilution by tributary inflow, but the total mass of AOX present in the water was fairly constant. During October 1989 about one third of the AOX mass disappeared from the water column along 240 km of the Wapiti-Smoky river system. During the winter surveys, there was no measurable loss of AOX from BKME over 240 km of the Wapiti-Smoky Rivers and over 1250 km of the Athabasca River. Supplementary sampling indicated that the AOX persisted even farther downstream, into the Peace and Slave rivers.

The chlorinated organic material accounting for AOX in BKME is thought to be 80 - 90% chlorinated lignin. Chlorolignin is fairly soluble and fairly resistant to decay, which is consistent with the high persistence of AOX observed here. Chlorolignin is fairly non-toxic, however, some researchers report that chlorolignin can degrade slowly to more toxic chlorophenolics.

1. INTRODUCTION

Adsorbable Organic Halide (AOX) refers to the amount of halide, principally chloride but also bromide and iodide, contained in organic compounds and recoverable during the analytical method. AOX has become an accepted measure of total chlorinated organic material, particularly that discharged by pulp mills using chlorine bleaching. Chlorinated organic material is of environmental concern because of the persistence and toxicity of some chlorinated organic compounds.

An AOX method has recently been implemented by the Alberta Environmental Centre, and is being used on bleached kraft pulp mill effluents (BKME) by Standards and Approvals Division (S&AD) of Alberta Environment to quantify chlorinated organic discharges. The Environmental Quality Monitoring Branch (EQMB) has initiated AOX sampling on rivers receiving BKME to assess the fate of such material. This report documents the results of receiving water sampling to date.

2. METHODS

Three surveys were carried out during which AOX was sampled:

Wapiti Smoky rivers - October 1989
- Feb-March 1990
Athabasca River - Feb-March 1990

These surveys progressed downstream at approximately the same speed as the river's time-of-travel and generally employed the methods described by Noton and Shaw (1989). Effluents, tributaries, and the mainstem rivers were sampled, and supplementary samples were also collected from the Peace and Slave rivers (Figure 1). Procter and Gamble Cellulose Ltd. discharges BKME near Grande Prairie and Weldwood of Canada Ltd. discharges BKME at Hinton. During both surveys, Procter and Gamble was employing about 25% ClO_2 substitution in its bleaching process to reduce chlorinated organic discharges and treating wastewaters in a 8-10d retention aerated lagoon. Weldwood was treating wastewaters in a 5-7d retention aerated lagoon and employing about 15% ClO_2 substitution. The pulp mills at Taylor, B.C. and Whitecourt do not use chlorine bleaching. In August, 1990, sampling again was conducted at some of the sites along the Athabasca River to obtain supplemental data.

Whole, unfiltered samples for AOX were collected in 500mL polyethylene bottles, acidified with nitric acid, and analyzed by the Alberta Environmental Centre, Research and Methods Development Branch. The analytical method (#E128.0; NAQUADAT #95080L) involves adsorption on activated carbon, combustion, and micro-coulometric titration, and is consistent with the Scandinavian Pulp, Paper and Board Testing Committee, Standard Method SCAN-W 9:89 (1989). The method is designed to measure both dissolved and particulate organic halogens. Trip (field) blanks were analyzed as per the method specifications. In addition, blind field blanks and blind duplicate samples were regularly submitted by the field

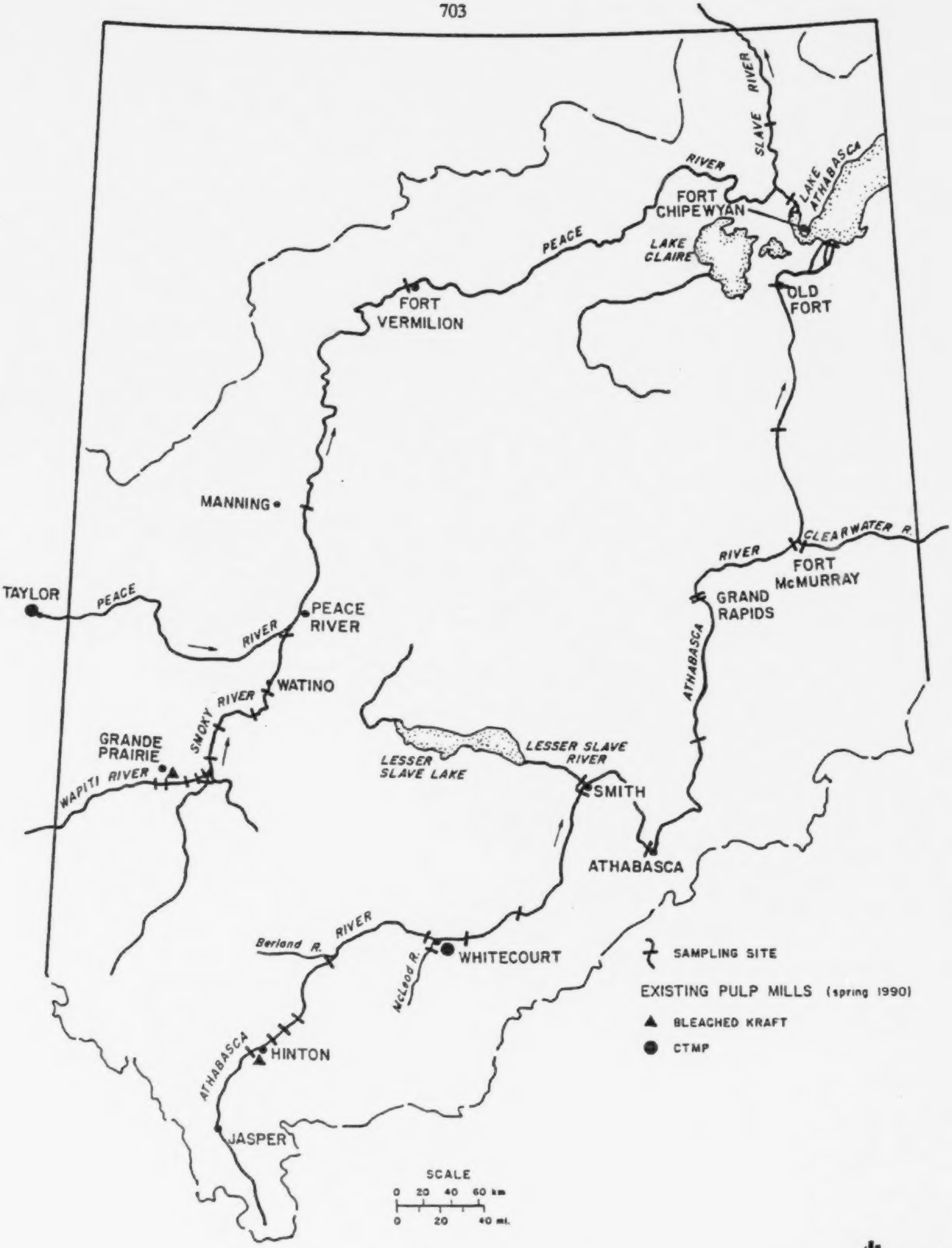
SLAVE RIVER
RIVER
LAKE ATHABASCA
FORT CHIPEWYAN
LAKE CLAIRE
PEACE
OLD FORT
FORT VERMILION
MANNING
CLEARWATER R.
RIVER
FORT McMURRAY
TAYLOR
PEACE
PEACE RIVER
RIVER
GRAND RAPIDS
ATHABASCA
WATINO
SMOKY RIVER
GRANDE PRAIRIE
WAPITI RIVER
LESSER SLAVE LAKE
LESSER SLAVE RIVER
SMITH
ATHABASCA
Berland R.
RIVER
McLeod R.
WHITECOURT
ATHABASCA
HINTON
JASPER
SAMPLING SITE
EXISTING PULP MILLS (spring 1990)
▲ BLEACHED KRAFT
● CTMP
SCALE
0 20 40 60 km
0 20 40 mi.

Figure 1. Sampling sites for AOX, 1989-90.

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crews. In August 1990, one sample from the Athabasca River was collected, mixed thoroughly in a stainless steel pail, split into eight replicates and analyzed to assess analytical precision.

Flows during the surveys were measured or estimated by Technical Services Division, Alberta Environment. Mass transport or 'load' of AOX was calculated as the product of concentration and flow, and expressed as kg/d, which is the unit used in effluent licencing: mg/L x m^3/s x 86.4 = kg/d.

3. RESULTS AND DISCUSSION

3.1 Quality Assurance

Field blank samples are checks on sample contamination from all potential sources (sampling, handling, laboratory), and AOX in such samples was low (0.000 to 0.004 mg/L, Table 1). Duplicate samples were submitted on 8 occasions and the percent difference within each sample pair was usually 0 - 4% (Table 1). Duplicate samples provide a check on all sources of variability that may affect the result. Analysis of the replicate split sample gave a standard deviation of 0.0014 mg/L at a concentration of 0.026 mg/L AOX. This is typical of the precision achieved with the method (I. Johnson, pers. comm.).

Flow measurements are also subject to errors, particularly during ice-covered conditions (G. Coles, pers. comm., Pelletier 1988) but replicate measurements to assess error are rarely done. However, since flow was measured sequentially downstream at approximately the rivers' time of travel, the flows can be summed to check their balance and provide an empirical assessment of accuracy. This is discussed below for each survey.

3.2 Wapiti and Smoky Rivers

During the synoptic survey of 2-5 October 1989, AOX was present at only trace concentrations in the upper Wapiti and upper Smoky rivers, but increased substantially in the Wapiti River downstream of the Procter and Gamble Cellulose Ltd. bleached kraft mill effluent (Table 2, Figure 2). The survey was carried out during near-average fall flows. Tributaries and other effluents contained insignificant loads of AOX. Mixing of the Wapiti with the Smoky diluted concentrations, but AOX was still present at the mouth of the Smoky, some 240 km downstream of the mill. The load calculations indicate that about one third of the AOX mass in the Wapiti downstream of the mill had disappeared from the water column by the mouth of the Smoky. However, there may be error in the load estimates since they also indicated about 20% greater load in the Wapiti than in the BKME. Such error probably results primarily from inaccuracy in flow measurements and travel time estimates.

During the winter survey, AOX was again negligible in the Wapiti River upstream of the pulp mill, in sampled tributaries, and in other effluents (Table 1). The bleached kraft mill discharged about 1450 kg/d

Table 1. Quality Control Samples for AOX

FIELD BLANK SAMPLES		DUPLICATE SAMPLES		
Date	AOX mg/L	Location & Date	AOX mg/L	% Difference from mean
		ATHABASCA SURVEYS		
14 Feb 90	0.000	AR u/s Hinton	0.000	0
8 Mar 90	0.002	14 Feb 90	0.000	
		Hinton Combined Effluent	3.3	3
		14 Feb 90	3.5	
		AR at Athabasca	0.030	9
		01 Mar 90	0.036	
		AR u/s of Horse R.	0.022	4
		14 Mar 90	0.024	
22 Aug 90	0.000	Hinton Combined Effluent	9.8	4
		21-22 Aug 90	10.7	
		WAPITI-SMOKY SURVEYS		
03 Oct 89	0.004	Procter & Gamble Effluent	23.1	2
		03 Oct 89	24.2	
06 Mar 90	0.001	Procter & Gamble Effluent	32	3
		27 Feb 90	33	
		Wapiti R. at Railroad Br.	1.3	0
		28 Feb 90	1.3	

REPLICATES

AR at Emerson Lakes Bridge - 22 Aug 90	AOX-mg/L	
	0.027	n = 8
	0.026	mean = 0.0260
	0.024	std. dev. = 0.0014
	0.024	coeff. var. = 5%
	0.028	
	0.027	
	0.026	
	0.026	

AR = Athabasca River

Table 2. Adsorbable Organic Halides (AOX) in the Wapiti, Smoky, and Peace River, 1989-90.

SITE	DISTANCE km d/s	OCTOBER 1989 DATE	FLOW m^3/s	AOX mg/L	AOX kg/d	FEB-MAR 1990 DATE	FLOW m^3/s	AOX mg/L	AOX kg/d
Wapiti R. @ Hwy 40	0	02 Oct	72.0	0.014	87	27 Feb	14.3	0.000	0
Grande Prairie STP Effluent	2	02 Oct	0.214	0.059	1.1	27 Feb	0.230	0.061	1.2
Wapiti R. d/s Haul Bridge	7.5	-	-	-	-	27 Feb	14.3	0.000	0
Procter & Gamble Storm Sewer/Cooling Water	9	03 Oct	(0.1)	0.052	0.4	28 Feb	0.048	0.000	0.02
Wapiti R. u/s of Mill Effluent	11	03 Oct	67.3	0.012	70	-	-	-	-
Procter & Gamble Final Effluent									
- ASB outflow	12	03 Oct	0.749	23.1 24.2	1490 1570	27-28 Feb -	0.516 -	32 -	1400 -
- Foam pond	12	-	-	-	-	28 Feb	0.516	33	1500
Wapiti R. @ Railroad Bridge	14	03 Oct -	72.1 -	0.29 -	1800 -	28 Feb	14.5	1.3 1.3	1600 1600
Wapiti R. u/s Bear R.	27	04 Oct	77.6	0.28	1900	28 Feb	14.5	1.4	1800
Bear R. mouth	27	04 Oct	2.23	0.034	6.5	-	-	-	-
Wapiti R. near mouth	44	04 Oct	80.6	0.27	1900	01 Mar	14.5	1.2	1500
Smoky R. u/s of Wapiti R.	43	04 Oct	106	0.004	40	01 Mar	24.7	0.003	6
Smoky R. @ Hwy 34	61	05 Oct	217	0.087	1600	06 Mar	61.1	0.34	1800
Smoky R. u/s of Puskwaskau R.	101	-	-	-	-	06 Mar	61.2	0.38	2000
Smoky R. @ km 105	142	-	-	-	-	07 Mar	64.8	0.34	1900
Smoky R. @ Watino	182	05 Oct	254	0.064	1400	07 Mar	81.0	0.31	2200
Smoky R. near mouth	246	05 Oct	279	0.056	1300	07 Mar	85.4	0.27	2000
Supplementary Sampling:									
Peace R. East of Manning	354	-	-	-	-	28 Feb 27 Mar	1061 1392	0.016 0.014	1500 1700
Peace R. @ Ft. Vermilion	682	-	-	-	-	28 Mar	1294	0.030	3600

( ) Estimated values

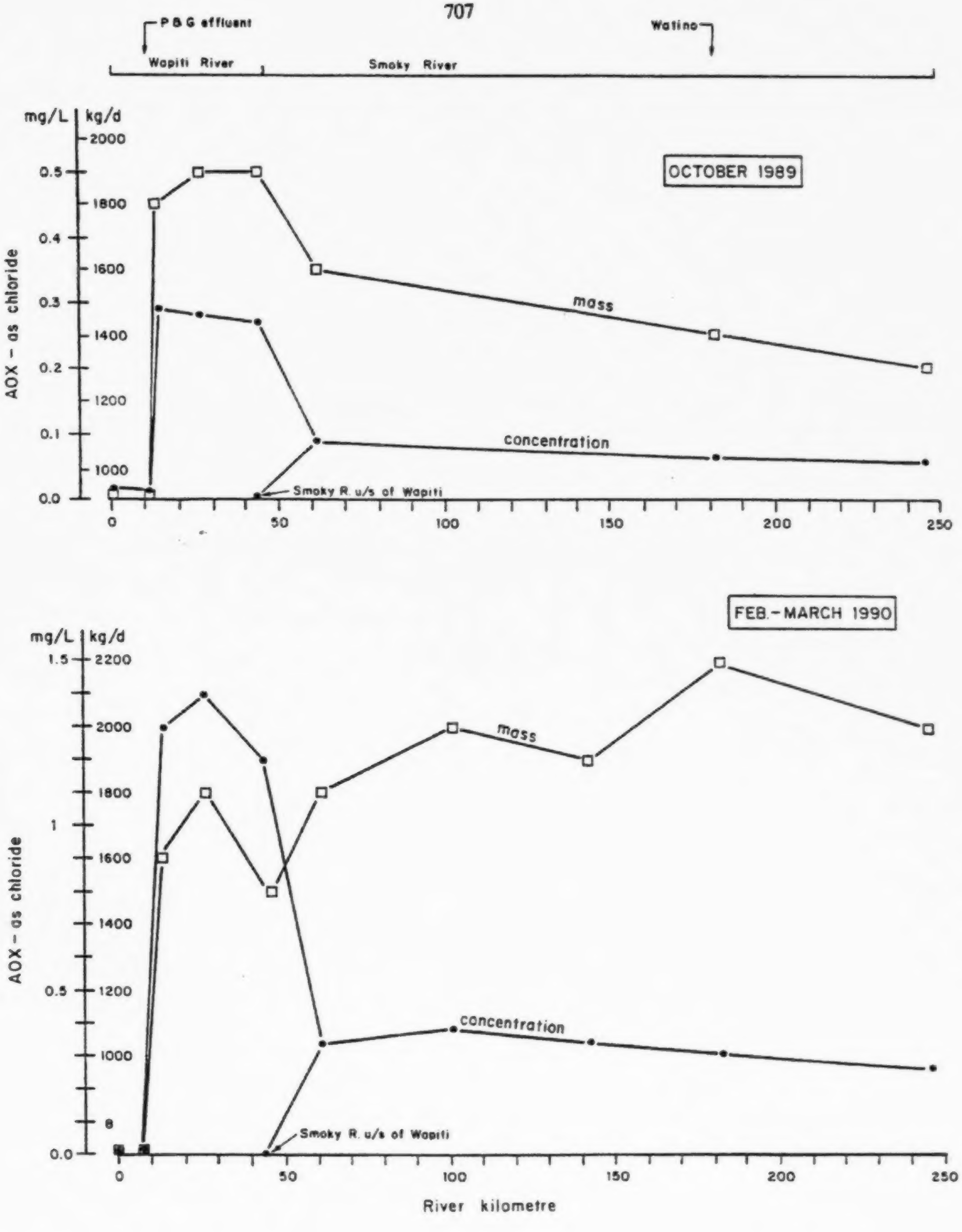

Figure 2. Adsorbable Organic Halides (AOX) in the Wapiti - Smoky Rivers, 1989 - 90.

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of AOX which markedly increased AOX concentrations in the Wapiti River (Figure 2). Concentrations declined substantially upon mixing with the Smoky River, and then declined slightly down the length of the Smoky. However, the mass of AOX in the water column did not decline (Figure 2), indicating that concentration declined due to dilution by tributaries. The AOX mass increased about 25% down the river during the survey. There was significant meltwater inflow at the time and this may have increased the errors in flow measurement and travel time estimates, and/or contributed additional AOX.

Supplementary sampling downstream in the Peace River revealed AOX concentrations of about 0.015-0.030 mg/L (Table 2). This indicates that chlorinated organic material originating from the kraft mill on the Wapiti River was detectable downstream in the Peace River, however, further investigation would be required to confirm this since sampling was not done at the rivers' time-of-travel (thus continuity of mass cannot be assumed), and since the Peace River upstream of the Smoky River was not sampled.

3.3 Athabasca River

In February 1990, AOX in the Athabasca River upstream of Hinton was not detectable, but increased to about 0.080 mg/L due to discharge of the Hinton Combined Effluent (HCE) (Table 3 and Figure 3). Concentration declined progressively downstream along the river, but AOX was still detectable at the river's mouth. The load of AOX in the water was negligible upstream of Hinton, but the HCE discharged about 230 kg/d to the river (Table 3). This mass remained in the water column throughout the length of the river (Figure 3). Note that the pulp mill at Hinton was shut down for part of February, 1990, and the AOX load in its effluent during this survey was lower than normal.

The load of AOX actually increased down the river, from about 230 kg/d near Hinton to about 300 kg/d in the lower reach of the Athabasca River. Other measured AOX inputs include (Table 3):

Other Effluents	5 kg/d
McLeod River	3
Lesser Slave River	26
Clearwater River	30
	64 kg/d

Unsampled tributaries accounted for about 107 m^3/s of flow during the survey. If it is assumed they contained AOX concentrations similar to the McLeod, Lesser Slave, and Clearwater Rivers (about 0.005 mg/L, Table 3), they would have contributed 46 kg/d, for a total of 110 kg/d of AOX input from sources other than the HCE. In view of the error to be expected in flow measurements, travel time estimates, and analytical procedures, this load of 110 kg/d likely accounts for the observed increase of 70 kg/d. Thus, about 75% of the AOX transported to Lake Athabasca during this survey appeared to originate from the Hinton combined effluent. AOX from the HCE showed no measurable disappearance

Table 3. Adsorbable Organic Halides (AOX) in the Athabasca River, Feb - March 1990

SITE	DISTANCE km d/s	DATE 1990	FLOW m^3/s	AOX	
				mg/L	kg/d
Athabasca R. u/s of Hinton	0	14 Feb	33.0	0.000 0.000	0 0
Hinton Combined Effluent (HCE)	1.9	15 Feb	0.784	3.3 3.5	220 240
Athabasca R. 6 km d/s of HCE	7.5	15 Feb	33.1	0.077	220
Athabasca R. 19 km d/s of HCE	23	15 Feb	33.4	0.089	260
Athabasca R. 36 km d/s of HCE	39	16 Feb	33.8	0.084	250
Athabasca R. u/s of Berland R.	104	16 Feb	36.0	0.074	230
Athabasca R. u/s of Whitecourt	210	20 Feb	50.7	0.047	210
McLeod R. @ mouth	211	20 Feb	10.1	0.003	3
Millar Western Effluent (CTMP mill)	212	20 Feb	0.136	0.12±	1.4
Whitecourt STP Effluent	215	20 Feb	0.039	0.11	0.37
Athabasca R. 10 km d/s McLeod R.	221	21 Feb	61.8	0.037	200
Athabasca R. @ Ft. Assiniboine	306	21 Feb	64.4	0.041	230
Athabasca R. u/s of Smith	438	28 Feb	73.3	0.047	300
Slave Lake STP Effluent		26 Feb	0.033	0.20	0.57
Lesser Slave R. mouth	449	01 Mar	43.7	0.007	26
Athabasca R. @ Athabasca	556	01 Mar	117	0.030 0.036	300 360
Athabasca STP Effluent	557	06 Mar	0.011	0.18	0.17
Athabasca R. near MacMillan Lake	678	06 Mar	123	0.026	280
Athabasca R. u/s Horse R.	946	14 Mar	142	0.022 0.024	270 290
Clearwater R. @ mouth	951	14 Mar	58.6	0.006	30
Ft. McMurray STP Effluent	951	14 Mar	0.180	0.16	2.5
Suncor Process Effluent	981	14 Mar	0.303	0.027	0.71
Athabasca R. near Bitumount	1029	15 Mar	207	0.019	340
Athabasca R. @ Old Fort	1157	21 Mar	218	0.014	260
Athabasca R. @ Lake Athabasca	1239	21 Mar	247	0.013	280
Supplementary Sampling:					
Lake Athabasca near Potato Island		21 Mar	-	0.020	
Riviere des Rochers		21 Mar	745	0.010	
Slave R. near laButte - LB		21 Mar		0.014	
- RB		21 Mar		0.016	
- mean			(2550)	0.015	

( ) Estimated value

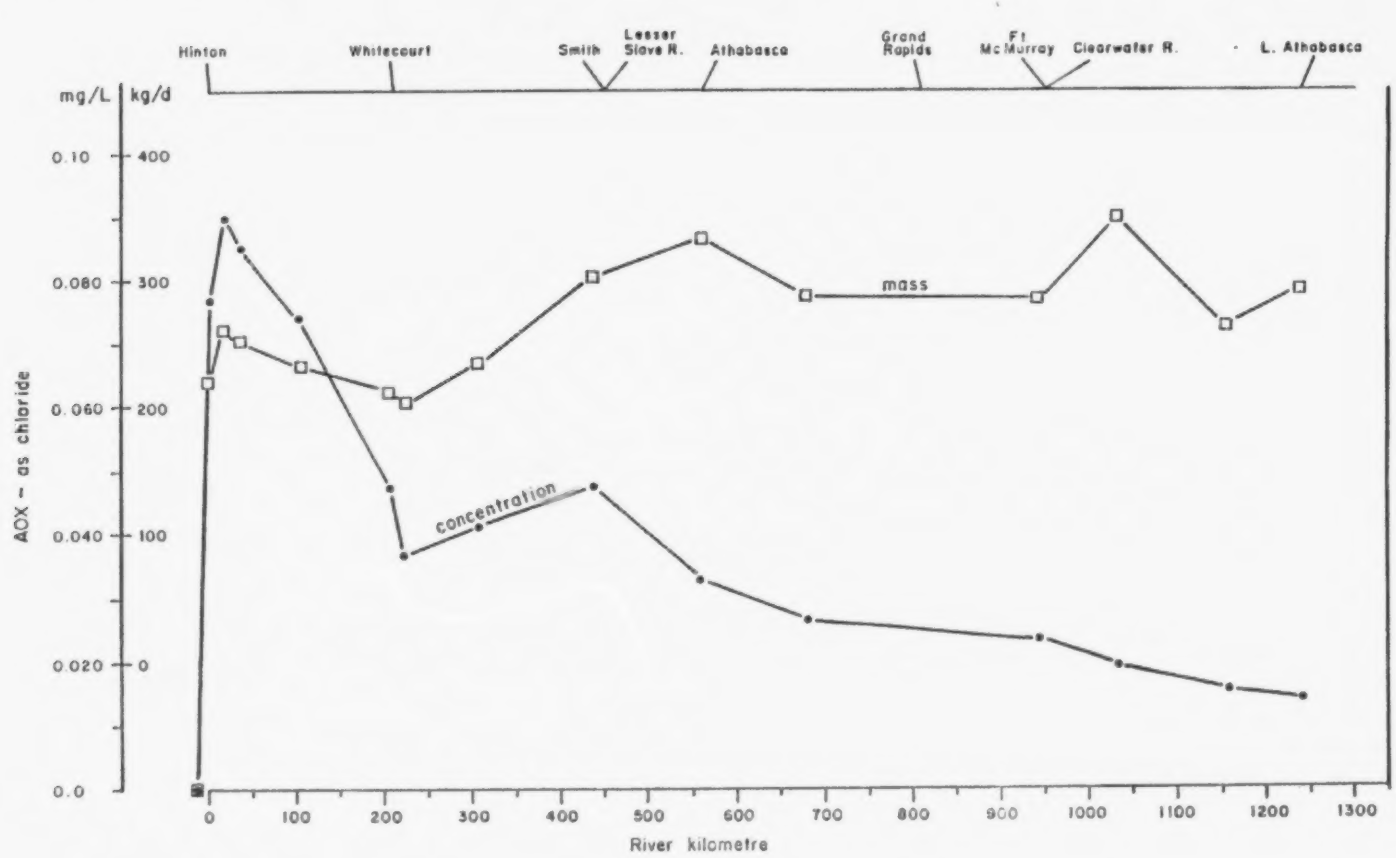

Figure 3. Adsorbable Organic Halides (AOX) in the Athabasca River, Feb. - March 1990.

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from the water column over that 1250 km distance.

Supplementary sampling farther downstream in Lake Athabasca and the Slave River (Table 3, Figure 1) revealed AOX concentrations similar to those in the lower Athabasca River (0.010 - 0.020 mg/L). This suggests the AOX persists farther downstream, however, more sampling would be required to confirm this since background levels of AOX in unaffected water bodies in that area are not known.

3.4 General Discussion

3.4.1 Nature of AOX

The identity of the organochlorine compounds from bleached kraft pulp mills effluents has been investigated by a number of researchers. It has been estimated that 80 - 90% of the organically bound chlorine (AOX) is in high molecular weight compounds (>1000 MW), generally referred to as chlorolignins (Salkinoja-Salonen *et al*, 1981; Kringstad and Lindstrom, 1984; Voss 1987). This material is fairly soluble and once discharged it appears to remain in the water column, as indicated by the findings here, and can serve as a tracer of BKME. AOX has been used as a tracer of BKME from Swedish mills on the Bothnian Sea (Sodergren 1989), although the researchers note that AOX can originate from sources other than BKME and therefore must be used as a tracer with caution. AOX from BKME will also include lower molecular weight compounds, some of which, are of environmental concern (e.g. chlorophenolics).

3.4.2 Background Conditions

The presence of measurable amounts of AOX at sites not influenced by BKME or municipal effluent suggests that there are naturally occurring organic compounds containing halogen atoms in surface waters. This has been reported elsewhere and the AOX concentration has been linked to the concentration of total organic carbon (TOC), and humic and fulvic acids (Wigilius *et al* 1988; Paasivirta *et al* 1988; Grimvall 1990). Background sites in this survey had the following AOX and TOC concentrations:

		AOX (mg/L)	TOC (mg/L)	AOX:TOC (mg/g)
Athabasca R. u/s Hinton	est.	0.0005	0.64	0.8
*McLeod R. mouth		0.003	4.43	0.7
*Lesser Slave R. mouth		0.007	11.0	0.6
Clearwater R. mouth		0.006	6.36	0.9
Wapiti R. u/s Grande Prairie	- Oct 89	0.014	6.57	2.1
	- Mar 90 est.	0.0005	2.57	0.2
Smoky R. u/s Wapiti R.	- Oct 89	0.004	5.9	0.7
	- Mar 90	0.003	1.6	1.9

*Receive Municipal sewage, but AOX apparently not affected.

The values are near the lower limit of detection, and are at the low end of background values reported by Wigilius et al (1988) of 0.010 - 0.050 mg/L and by Martinsen et al (1988) of 0.010 - 0.015 mg/L. The mean AOX:TOC ratio of about 1 mg/g is also at the low end of background values reported by Wigilius et al (1988) and Grimvall (1990) of 1 - 5 mg/g and 0.7 - 8.6 mg/g, respectively.

3.4.3 Persistence

The findings here indicate no measurable loss of AOX from BKME in the two receiving river systems during winter, over the length of river investigated. The distance from Hinton to Lake Athabasca is about 1250 km and the travel time was about 35 days. During the fall survey, there was an indication of some disappearance of AOX over the 240 km of the Wapiti-Smoky river system (travel time about 4 - 5 days), however, further investigation would be necessary to confirm the loss. The downstream persistence of AOX in winter in the Wapiti-Smoky mirrors the persistence reported there for organic carbon, colour, and phenolics from the BKME (Noton et al 1989). Conditions during the winter surveys included water temperatures of 0°C, ice cover, and low suspended solids (1 - 5 mg/L), whereas during the fall survey water temperatures were about 7 - 9°C and suspended solids about 20 - 45 mg/L. Differences in these and other variables may be related to the apparent downstream loss of AOX in the fall.

No studies of AOX persistence in receiving rivers could be found in the scientific literature, although persistence of AOX in the Gulf of Bothnia (Sodergren 1989) and of other pulp mill effluent constituents in receiving waters (e.g. Brownlee and Strachan 1976; Merriman 1988) have been reported. Chlorophenols attributable to bleached kraft pulp mills on the upper Fraser River have been detected in the Fraser River Estuary, some 500 km downstream (Carey and Hart, 1988). A March 1990 sampling of the lower Athabasca River, downstream of Ft. McMurray, revealed chlorophenols to be present in the ng/L range (B. Brownlee, pers. comm.). The probable source of these compounds is the bleached kraft mill at Hinton, about 1000 km upstream.

3.4.4 Environmental Significance

Although AOX standards for pulp mill effluents are being set in several jurisdictions, no surface water quality objectives exist for the sum parameter AOX. Most of the AOX from BKME is probably chlorolignins, which because of their high molecular weight cannot penetrate cell membranes and exert toxicity (Salkinoja-Salonen et al 1981; Kringstad and Lindstrom 1984, Sagfors and Starck 1988). However, chlorolignin imparts colour to water which may have aesthetic impact and which may reduce light penetration and photosynthesis. As well, chlorolignin has been demonstrated to undergo biochemical breakdown under environmentally relevant conditions to more toxic chlorophenolic compounds, albeit at relatively slow rates (Erikson and Kolar 1985; Erikson et al 1985). Even though downstream disappearance of AOX was not observed in the winter surveys, such breakdown or biotransformation may be occurring: the resolution of the AOX measurement (µg/L) may be too gross to detect such changes (ng/L) and/or the breakdown products may also remain in the water column and be measured as AOX (e.g. soluble chlorophenolics).

4. ACKNOWLEDGEMENTS

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CHARACTERISTICS OF FISH POPULATIONS IN A LAKE SUPERIOR BAY RECEIVING BLEACHED KRAFT MILL EFFLUENT.

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Extended Abstract

The impact of bleached kraft pulp mills on aquatic ecosystems is a matter of international concern. Andersson et al. (1988) reported a survey of bleached kraft mill effluent (BKME) impacts on perch (*Perca fluviatilis*) collected off the east coast of Sweden. Perch exposed to BKME exhibited physiological changes up to 8 to 10 km from the effluent disposal site. The major changes consisted of decreased gonad growth, increased liver size, a ten-fold increase in liver MFO activity, abnormal carbohydrate metabolism, changes in haematology and increased fin erosion. Although their investigation of physiological and biochemical impacts was extensive, the study neglected to tie the changes together and offer any mechanism to explain the impacts. The content, design and conclusions of the Scandinavian studies have been challenged (Sprague and Colody, 1989). Our 1988 and 1989 collections parallel those of the Swedish studies.

Up until the initiation of secondary treatment in October of 1989, Jackfish Bay, Lake Superior, had received the primary-treated effluent from a bleached kraft mill for several decades. During 1988 and 1989, white sucker were collected from Jackfish Bay, and from Mountain Bay, which served as a reference site. White sucker (*Catostomus commersoni*) were collected from the spawning runs in Sawmill Creek (Jackfish Bay) and the Little Gravel River (Mountain Bay) by overnight hoop net sets. During the summer months, the fish were collected by gillnet. Steroids were measured by radioimmunoassay by standard methods; MFO determinations were conducted by Ontario Ministry of Environment personnel.

During all collections, male and female white sucker exhibited a decreased length and weight and a higher condition factor at the BKME site. The increased condition factor and body fat were consistent with an increase in food abundance and water temperature at the BKME site, but were inconsistent with impacts on growth and reproduction. Both sexes exhibited a decreased growth rate and a shift in size distribution towards smaller fish. Males exhibited an age distribution pattern consistent with decreased longevity (increased mortality) or missing year classes. Female fish had a decreased egg size and decreased gonadal weight, and egg size at the BKME site did not increase with age. During the August collections, ovaries were much smaller at the BKME site than at the reference site, and male fish showed no secondary sexual characteristics (tubercles). All mature males examined in August at four other sites showed significant tubercle development.

The MFO analyses determined the activity of benzo(a)pyrene (BaP) hydroxylase (aryl hydrocarbon hydroxylase activity, AHH) towards diphenyloxazole (PPO) and BaP. Summer-collected white sucker showed a 6- to 18-fold increase in activity towards PPO, and 5-fold increase in BaP activity. There was no significant difference in UDPGT activity (glucuronosyl transferase activity towards p-nitrophenol) in the summer samples, which is consistent with previous findings of other investigators. During 1989, spawning male white sucker showed a 2- to 3-fold increase in activity for both BaP and PPO substrates (AHH activity). Spawning females did not show increased activity relative to the reference site, consistent with the 1988 collections, and with previous studies which have reported a shut-down of MFO enzymes at spawning time.

Testosterone levels were significantly reduced in both prespawning and spawning females at the BKME site. Testosterone, 11-ketotestosterone and $17\alpha,20\beta$ dihydroxyprogesterone levels were significantly reduced in exposed males during the prespawning and spawning periods. July samples showed no differences in female steroid levels, but males showed reduced levels of testosterone and 11-ketotestosterone; August samples showed reduced testosterone in both females and males relative to two reference sites, as well as reduced 11-ketotestosterone levels in males and reduced 17β-estradiol levels in females.

Ripe male white sucker collected during 1989 showed decreased spermatocrit and sperm motility but no difference in milt volume or seminal plasma constituents (Na, K, Cl, osmolality). Samples collected in 1989 confirmed the decreased egg size observed in 1988, but there was no apparent difference in fertilization rates or egg release. Studies on eggs returned to the laboratory showed no differences in development, behaviour or initiation of exogenous feeding (M.E. McMaster, unpubl. data).

Although condition factor increased, there was no concomitant increase in growth rate, suggesting that factors associated with energy allocation may have been affected. The white sucker exhibited an increased condition factor and increased peritoneal fat deposits. The combination of increased condition factor, decreased growth (length at age), and decreased fecundity observed at the BKME site appears paradoxical. Fish which cannot obtain sufficient energy for either growth or reproduction would be expected to show a lower condition factor. These results are consistent with the hypothesis of impacts of increased MFO activity on factors regulating carbohydrate metabolism.

During 1989, whitefish collected from under the BKME plume were found to have a marked elevation in body fat, decreased gonadal size and increased liver weight relative to those at two reference sites. Whitefish collected under the plume are also very sensitive to stress and seldom survived net collection. This phenomenon is not apparent at the reference sites. The consequences of these findings for whitefish and other species in the area are unknown. Changes in the fish populations following the installation of an aerated stabilization basin are continuing.

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THE IMPACT OF TWO GREAT LAKES BLEACHED KRAFT MILLS (BKME) ON RECEIVING WATER MUTAGENICITY AND THE BIOCHEMISTRY AND PATHOLOGY OF WILD WHITE SUCKERS INHABITING IMPACTED AREAS.

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Extended Abstract

Concern about non-traditional pollutants discharged by bleached kraft mill operations (BKME) has prompted investigations of a range of sub-lethal impacts in biological systems. Two areas of potential concern include the discharge of chemicals which induce hepatic mixed function oxidases (MFO's), and the discharge of mutagenic and hence potentially carcinogenic chemicals. MFO induction by BKME may be associated with reproductive disorders (Munkittrik et al this volume) and in fish exposed to a BKME and its mutagenic components (Douglas et al 1979) may exacerbate the impact of such contaminants.

Between 1985 and 1988 the Ministry of the Environment investigated two BKME operations adjacent to Lake Superior. Mill A included both a sulphite and bleaching type operation, while Mill B only discharged BKME. The AMES fluctuation test was utilized to assay the mutagenicity of the receiving water in areas which might be inhabited by fish. The fluctuation test can accomodate up to 75% v/v (15 mL/20mL total volume) water or effluent, and is a sensitive assay for dilute mutagens without the complications of concentration steps. Positive responses were obtained with 5 mL (1985) and 15 mL (1986) of receiving water 100 m. below Mill A but only 2.5 mL of the water 500 m. below Mill B (both 1986 and 1987) was needed to induce mutations. It is difficult to compare these results without defining mixing zones and dilution/production rates, however the AMES fluctuation test clearly defined a zone of potential mutagenicity.

White suckers were captured in 1986 and 1988 from these sites for studies of hepatic MFO's, the Phase II conjugation enzyme Uridine-5'-diphosphoglucuronic acid transferase (UDPGT), and skin and liver cancer. MFO induction and UDPGT depression have been observed in several Scandanavian studies to occur for considerable distances from the mill outfall (see for example Lindstrom-Seppa and Oikari 1990). MFO (2,5-diphenyloxazole oxidation) induction (5 and 7 fold) and UDPGT (p-nitrophenol conjugation) depression (60% and 50%) was observed in our studies at both mill sites.

Skin cancer has been reported to affect white suckers throughout the Great Lakes, and it is likely caused by an oncogenic (carcinogenic) virus. Under some circumstances however an increased prevalence in polluted areas may indicate a co-carcinogenic effect of pollution and the virus (Smith et al 1989). Skin cancer affected 2.8% (N=139) of the white suckers inhabiting the impact acreas of Mill A compared to a rate of 11.0% (N=200) in a reference area. The population below Mill B however had an incidence of 8.5% (N=200) versus a reference rate of 4.5% (N=200) in the spring, and a rate of 2% (N=100) versus 1% (N=100) in summer samples. Whether BKME is co-carcinogenic for skin cancer in white suckers remains equivicable, because the background rate of this viral disease may range up to 20% (Smith et al 1989).

Histopathological examination of the livers of white suckers found a degenerative and proliferative condition centred about the bile ducts. This disease exists in various degrees throughout the Great Lakes, raising suspicions that is is induced by either parasites or some widespread contaminant, but it is more severe (including neoplasms) in areas contaminated with industrial pollutants (Hayes et al 1990). This condition affected 21% of the suckers captured in the summer below Mill A, compared to 6.3% in a reference population. While 65% of the suckers taken in the spring at a spawning stream adjacent to Mill B had this condition, the reference population also was markedly (78%) affected. Bile duct neoplasms (cancers) were found in 2% and 6% of suckers from locations A and B (N=139 and 117). No neoplasms were found at the reference site for Mill A, but a single neoplasm (1% incidence) was found at the Mill B reference. The spawning reference population for Mill B may include suckers which have migrated from the plume of another pulp-and-paper operation 10 miles away, however during MFO studies suckers were resident in the river mouth and were not affected.

The possible induction of liver neoplasms by BKME has not been reported previously in Scandanavian or other locations. The degenerative bile duct disease which affects white suckers (and also brown bullheads) in the Great Lakes is clearly a pre-disposing factor for the development of liver neoplasms; as such other species which do not exhibit this condition may not be at carcinogenic risk.

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SUMMARY

For the first time since 1971, Canada is revising its guidelines to limit the discharge of pulp and paper wastes. Incentive for change came from reviews of in situ studies in Scandinavia of effects of Bleached Kraft Mill Effluents on fish (Sprague and Colodey 1989). Biochemical and physiological responses of fish typical of exposure to BKME were associated with changes in population structure and productivity (Table 1). Chemical contamination by chlorinated compounds was strongly linked to enzymatic changes in liver typical of chemical exposure and metabolism. Metabolites of phenolics were excreted in bile and the livers of fish were swollen. There were variable effects on ion and energy regulation but consistent hematological responses indicative of stress and direct toxicity. The prevalence of skeletal deformities and fin erosion were elevated, and sexual maturation was retarded in adult fish. There were associated shifts in the abundance of fish, species composition, recruitment and year-class success.

These studies indicated important impacts of BKME on fish health and productivity. While some results were variable, dose-response relationships were obvious based on dilution gradients, studies of caged fish, and laboratory experiments. Negative responses were often understandable in terms of local exposure patterns.

While these studies suggest that chlorinated compounds in BKME should be controlled, there was skepticism as to whether they applied to Canada (Sprague 1989). The Scandinavian data are confounded by other sources of chemicals and there were no equivalent Canadian field studies (Kovacs 1986). Some laboratory studies (e.g. McLeay and Brown 1979) indicated that BKME affects energetics of fish, but the detailed studies of detoxication enzymes were not replicated. Canadian data were primarily from isolated studies of chemical distribution, presence/absence of fish species and impacts of organic enrichment and low dissolved oxygen on benthic invertebrates (Kovacs 1986; McLeay 1987). The only multidisciplinary study, that of BKME discharged to Nipigon Bay, Lake Superior, did not link biochemical and physiological responses of individual fish to performance of populations (Kelso 1977; Whittle and Flood 1977).

Therefore, we studied populations of white sucker (Catostomus commersoni) 10 km upstream and 2, 35 and 96 km downstream of a bleached kraft mill at LaTuque, Quebec on the St. Maurice R. The mill's effluent contains about 10 kg of chlorinated compounds per air-dried tonne of product, with a daily production of 1200 Tonnes, and an initial effluent dilution of 1:93. Further details of the effluent are presented by Carey (this issue). The St. Maurice forms a natural laboratory, in that a dam at LaTuque separates the upstream control site from the downstream sites, and a second dam 110 km further downstream defines a stretch of river with no other source of pollution and no entry of fish from downstream areas of low water quality. The time of flow of the river and a few

Table 1. Summary of results of studies in Scandinavia[1] and Quebec on BKME effects on fish. '+' = increase; '-' = decrease; 'o' = no change.

RESPONSE			SCANDINAVIA	QUEBEC
Contamination by Chlorinated Compounds				
	Resin acids and phenolics		+	
	Extractable organic chlorine		+	
	Dioxins		+	+
Chemical Metabolism				
	MFO induction	- EROD	+,o	+
		- ECOD	+,o	
		- AHH	+,o	+
		- Cytochrome P-450	o	
	UDP Glucuronosoyl transferase		+,o	
	Glutathione-S-transferase		o	-
	NADPH cytochrome-c-reductase		o	
	Glucuronide conjugates of phenolics in bile		+	
	LSI		+	+
Ionoregulation				
	Plasma	Na	+,o	
		K	-	
		Mg	+,o,-	
		Cl	o,-	
		Ca	+,-	
Hematology				
	Hct, Hb, RBC count, MCH		+,-	+
	RBC vol		+	
	ALA-D activity		+	
	Methemogoblinemia		+	
	Bilirubin		+	
	Plasma protein		-	+
	WBC numbers	lymphocytes	-	
		neutrophils	+	
		thrombocytes	+,-	
Energetics				
	Muscle glycogen		+	
	blood glucose		+,o,-	+
	blood lactate		+,o	
	liver ascorbic acid		+	
	liver glycogen		+	
	CF		o	o
	GSI		-	-
Pathology				
	vertebrae	deformities	+	+
		collagen	-	
		hydroxyproline	+	
		mechanical properties	+	
	Fin erosion		+	
Population responses				
	Abundance		+,-	
	Species shifts		+	
	Recruitment		-	
	Age at Maturity		-	-

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[1] Information extracted from the following references: Andersson et al. 1987; 1988; Bengtsson et al. 1988a; 1988b; Hardig et al. 1988; Larsson et al. 1988; Lindstrom-Seppa et al. 1989; Lindstrom-Seppa and Oikari 1989; Matssoff and Oikari 1988; Nikinmaa and Oikari 1982; Oikari 1986; Oikari and Anas 1985; Oikari and Holmbom 1986; Oikari et al. 1983; 1984; 1985; Oikari and Niittyla 1985; Oikari and Kunnamo-Ojala 1987; Sandstrom et al. 1989; Tana and Nikuna 1986.

small tributaries provide a natural gradient of biodegradable compounds as described by Carey (this issue). Biases in the study include log driving, deposits of bark, wood and wood fibre at all sites including control, and the entry of the effluent immediately upstream of the dam that separates the control from treated areas. However, the strong current in the river appears to entrain the effluent in the discharge from the dam, and the control site is 8-10 km upstream of the effluent source. The data described here were primarily from a preliminary study in 1989 when sample sizes varied from 15 at the control site to 8 at the site furthest downstream.

Using the Scandinavian studies as a guide, we measured:

- chemical contamination: chloroguaiacols in water as an indicator of the presence of BKME (Carey, this issue); dioxins/furans in fish;
- Metabolism of chemicals in liver: Aryhydrocarbon hydroxylase (1989); ethoxyresorufin-o-deethylase (1990); glutathione-s-transferase; liver somatic index;
- stress: hematocrit; serum glucose; serum protein; size; condition factor;
- sexual maturation: gonad somatic index; serum testosterone, 11-ketotestosterone and estradiol.

Measurements in August 1989 (Table 1) indicated chemical exposure of fish. Tetrachloroguaiacols in water decreased in a linear fashion from their source to the site furthest downstream, describing a gradient of exposure to compounds that degrade (Carey, this issue). Dioxins and furans were present in whole fish, but showed no declining trend down the river, implying either a homogenous distribution or inadequate sample sizes. Since these compounds are much more persistent than phenolics, the former idea is most likely.

Chemical contamination was associated with a 10-fold increase in AHH activity from control to treated sites, followed by a 'decay' of enzyme induction towards normal at the most downstream sites. While this pattern of response implied a declining exposure of fish with distance downstream, levels of MFO activity remained 5 times higher than control 100 km downstream. Liver somatic index showed a similar pattern of response, with an increased liver size positively correlated to enzyme activity and implying enzyme induction. Hematocrit, serum glucose and serum protein also followed the same pattern, and all changes were statistically significant with the exception of serum protein. We call this pattern of response the 'effluent' effect, since it follows the pattern of effluent contamination and dilution. It reflect chemical exposure and stress, although the stress was not expressed in significant changes in condition factor.

For measures of sexual maturation, a different pattern emerged. Relative to the control site, there were no changes in gonad somatic index at the first site downstream, suggesting no direct impact of the effluent. However at 35 and 100 km downstream, there was a progressive decline in gonad somatic index, especially of males. Coincidentally, there were increases in the levels of serum estradiol and testosterone in females. We have termed these effects the 'downstream' effect, since they increased with distance from the source of the BKME. However, levels of male hormones followed the effluent effect and we are unable to explain this apparent contradiction between GSI and hormone levels of males with the limited data from 1989. The decline in GSI was associated with an increase in the fattiness of fish, as judged visually from peritoneal fat deposits. Since this was also evident in other species, it supports the idea of a downstream effect. The cause is unknown, but may be related to the transformation and sediment contamination processes described by Carey (this issue).

In summary, we observed important changes in the fish of the St. Maurice, similar to those observed in Scandinavian fish and typical of exposure to BKME. While there are some contradictory findings associated with very small sample sizes, there is a strong relationship between chemical exposure and impacts on fish, and we are currently studying the river more intensively to better understand the responses of fish populations and the underlying mechanisms. Studies in 1990 have already confirmed the MFO response and we have added pathology to the list of measurements being made.

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Effects of pulp and paper mill effluents on estuarine and marine environments in Canada: A brief review

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ABSTRACT

Pulp and paper mill effluents from Canadian coastal mills are usually acutely toxic at source, and in many cases have marked deleterious effects on receiving waters due to toxicity, high BOD and TSS loadings. Extreme reductions in ambient dissolved oxygen, impacts on benthic and intertidal organisms, changes in water colour and primary productivity, and contamination of biota by a wide range of organochlorine compounds, have been demonstrated.

Where BOD has been treated successfully, dissolved oxygen levels have improved. However, even in these cases, long-term impacts of large accumulations of bark and effluent derived solids on inshore benthic habitats are expected. While sublethal effects of lowered dissolved oxygen levels and suspended solids on the water column and bottom communities are well known, the potential effects of major organochlorine contamination of water, sediments and biota are not fully understood, especially under natural and perturbated conditions. The findings of recent North American and Scandinavian studies which describe liver enzyme activation, reproductive changes and histological damage in fish, are a major concern. Site-specific assessments need to be updated in light of current biomonitoring techniques and changes at mill sites.

Although this review was undertaken using 1987-88 data that has been superceded by vast changes to mill in-plant and treatment processes, the environmental effects described herein are largely long-term problems which will not respond quickly to changes in pollutant loading.

INTRODUCTION

This review describes the serious environmental effects of pulp and paper mill effluents discharged to estuarine and marine waters in Canada. In recent years attention has shifted away from traditionally regulated parameters like BOD (Biochemical Oxygen Demand), TSS (Total Suspended Solids), and toxicity (as measured by a 96 h trout bioassay), to focus on the impact of dioxins-furans and other organochlorines, when these compounds were brought into public focus following fisheries closures related to dioxin and furan contamination (Sprague and Colodey 1989, Whittle et al. 1990).

The loading, fate and impact of each federally regulated parameter is addressed, and the effects of organochlorine substances present in some effluents are described. Information on freshwater impacts has been included to illustrate potential marine and estuarine impacts which should be evaluated. Although this review was undertaken using 1987-88 Environmental Portection (EP) data that has been superceded by on-going changes to mill process and treatment works, the environmental effects described herein are largely long-term problems which will not respond quickly to changes in pollutant loading.

Overview of Inputs and Impacts

In general west coast mills are larger, integrated mills which use more water, have higher BOD and TSS loadings, and higher toxicity emission rates than east coast mills. (Tables 1 & 2). Impact is not always proportional to production and

TABLE 1 EFFLUENT AND PRODUCTION DATA FOR B.C. COASTAL MILLS RANKED BY INCREASING PRODUCTION[1]

MILL LOCATION	BOD $t \cdot d^{-1}$	TSS $t \cdot d^{-1}$	TSS $kg \cdot t^{-1}$	LC50 %	FLOW $m^3 \cdot 10^3 \cdot d$	TER[2]	PRODUCTION $t \cdot d^{-1}$
Port Alice[3]	85	4	8.7	33	175	530	462
Woodfibre	24	11	17.7	11	76	691	620
Port Mellon	16	7	10.8	24	117	488	647
Gold River	19	9	12.9	54	147	272	699
Harmac	22	9	8.6	75	265	353	1043
Prince Rupert	37	12	10.9	11	182	1655	1103
Port Alberni	9	10	7.7	64[4]	209	327	1294
Powell River	28	29	16.6	59	320	542	1751
Crofton	44	19	9.9	30	230	767	1910
Elk Falls	52	25	12.2	23	250	1087	2039
TOTAL	336	135	-	-	1971	6711	11568
RANGES	9-85	4-29	7.7-17.7	11-75	76-320	272-1655	462-2039
MEDIAN	26	10.5	10.9	32	196	536	1073

1 Environment Canada, Pacific & Yukon Region 1987 Data

2 Toxicity Emission Rate Calculated by $(100 \cdot LC50^{-1}) \cdot FLOW$ (see McLeay 1987)

3 Sulphite Mill

4 4/5 Tests Passed LC50 of 80%

TABLE 2
EFFLUENT AND PRODUCTION DATA FOR THE ATLANTIC PROVINCES COASTAL MILLS[1] RANKED BY INCREASING PRODUCTION

MILL LOCATION	BOD	TSS	TSS	AOX	LC50	FLOW	TER[2]	PRODUCTION
	t·d-1	t·d-1	kg·t-1	t·d-1	%	$m^3 \cdot 10^3 \cdot d^{-1}$		$t \cdot d^{-1}$
Hantsport, N.S. (CKF Inc.)	N/A	<1	--	N/A	>100	N/A	--	40
Saint John, N.B. (Irving Tissue)	N/A	<1	--	N/A	>100	N/A	N/A	50
Utopia, N.B.	17	2	6.7	N/A	12	6	50	300
Nelson, N.B.	2	1	3.3	N/A	2	7	350	300
East River, N.S.	4	4	12.9	N/A	19	3	16	310
Atholville, N.B.	20	3	8.8	2	20	60	300	340
Stephenville,Nfld	7	3	6.7	N/A	9	33	370	450
Abercrombie, N.S.	5	3	5.3	1	>100	84	<84	570
Brooklyn, N.S.	29	7	11.5	N/A	30	41	137	610
Bathurst, N.B.	35	5	6.9	N/A	32	40	125	730
Saint John, N.B. (Irving Pulp & Paper)	19	10	13.3	2	6	77	1280	750
Corner Brook, Nfld	17	30	39.0	N/A	20	94	450	770
Newcastle, N.B.	5	13	16.5	3	>100	65	<65	790
Saint John, N.B. (Rothesay Paper Inc.)	18	15	17.1	N/A	6	45	750	880
Point Tupper, N.S.[3]	52	19	20.7	2	2	87	4350	920
Dalhousie, N.B.	24	12	13	N/A	20	61	305	930
TOTALS	254	127	--	10	--	703	8632	8740
RANGES	2-52	<1-30	3.3-39	1-3	2->100	3-94	<65-4350	40-930
MEDIAN	17	4.5	10.9		20	43	221	590

[1] EP, Atlantic Region, 1988, Pulp and Paper Mills Sector Report

[2] Toxicity Emission Rate Calculated by $(100 \cdot LC50^{-1}) \cdot FLOW$ (see McLeay 1987)

[3] Sulfite Mill

waste loading: some large production mills with high loadings are located in areas with high assimilative capacity and cause little environmental degradation, while other small production mills with high waste loadings cause extensive habitat deterioration.

The locations of the ten mills discharging to estuarine and marine areas on Canada's west coast are depicted in Figure 1. Measurable impact has been documented at every coastal mill, although the magnitude of these impacts varies considerably. Changes in mill process (e.g. sulfite recovery, or switching from sulfite to kraft) and changes in discharge mode (from surface outfall to submerged diffuser) have led to environmental improvement at many locations (Kay 1989). In contrast, other changes such as increased loading due to mill production increases, cumulative impact or reduced assimilative capacity via natural factors (such as reduced river runoff or tidal exchange) are threatening fishery resources at other sites (eg. Colodey et al. 1988.) Mill locations in the Atlantic Provinces are shown in Figure 2. Measurable impacts have been documented at eight mills. Most of the mills are older ones, that did not provide treatment for their effluents prior to discharge (Waldichuk 1988a).

Along the St. Lawrence River and Estuary, 29 mills were in operation in 1982 and collectively discharged about 703 $t \cdot d^{-1}$ of BOD and 336 $t \cdot d^{-1}$ of TSS, a 50% decrease from TSS levels discharged in 1973. In the Saguenay Fjord, pulp wastes have been detected through measures of enhanced organic material in the sediments and have provided a historical record of the industry's activities (Smith 1988).

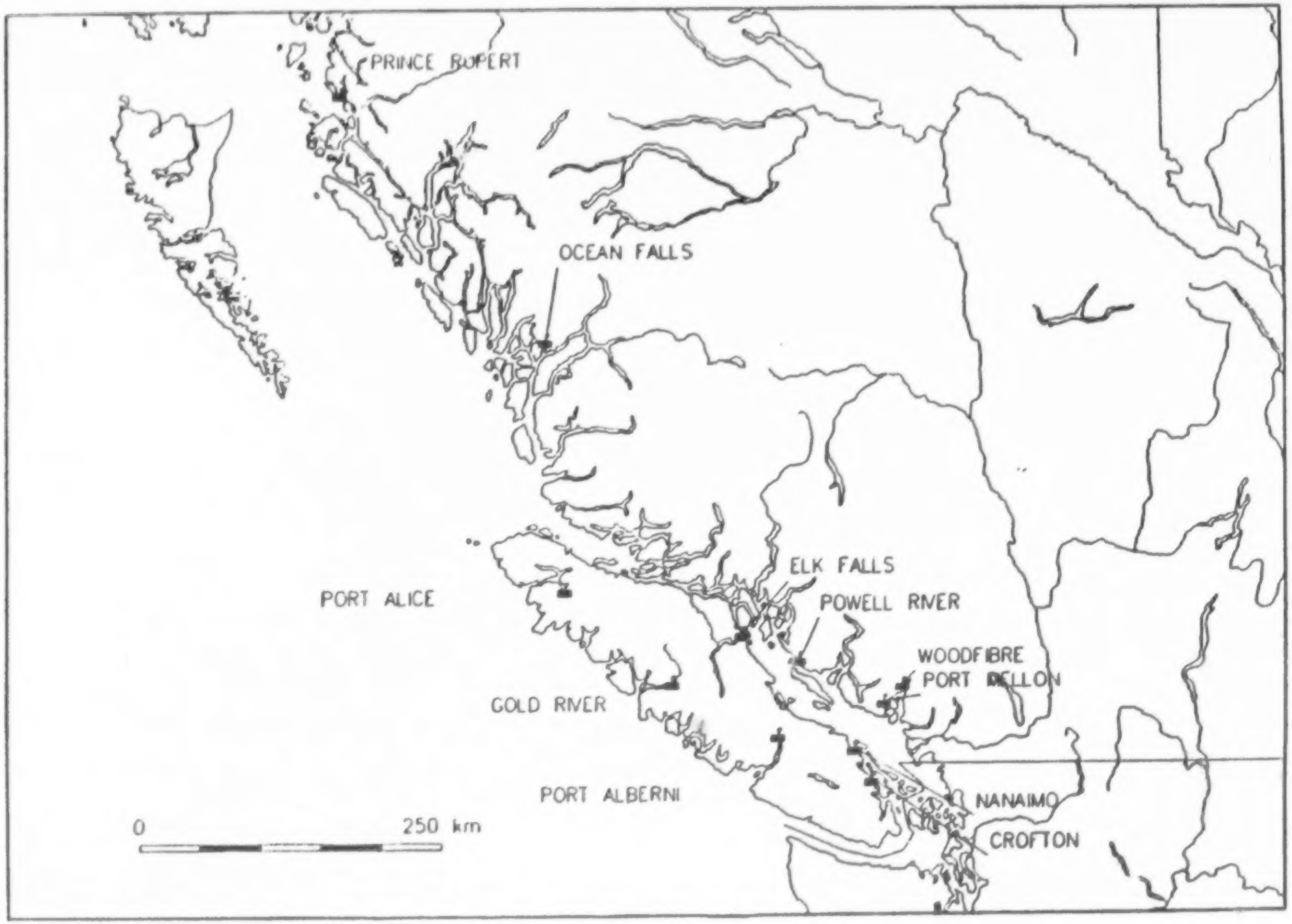

PRINCE RUPERT
OCEAN FALLS
ELK FALLS
PORT ALICE
POWELL RIVER
WOODFIBRE
PORT MELLON
GOLD RIVER
PORT ALBERNI
NANAIMO
CROFTON
0
250 km

FIGURE 1

LOCATION OF MILLS DISCHARGING TO ESTUARINE AND MARINE AREAS ON CANADA'S WEST COAST

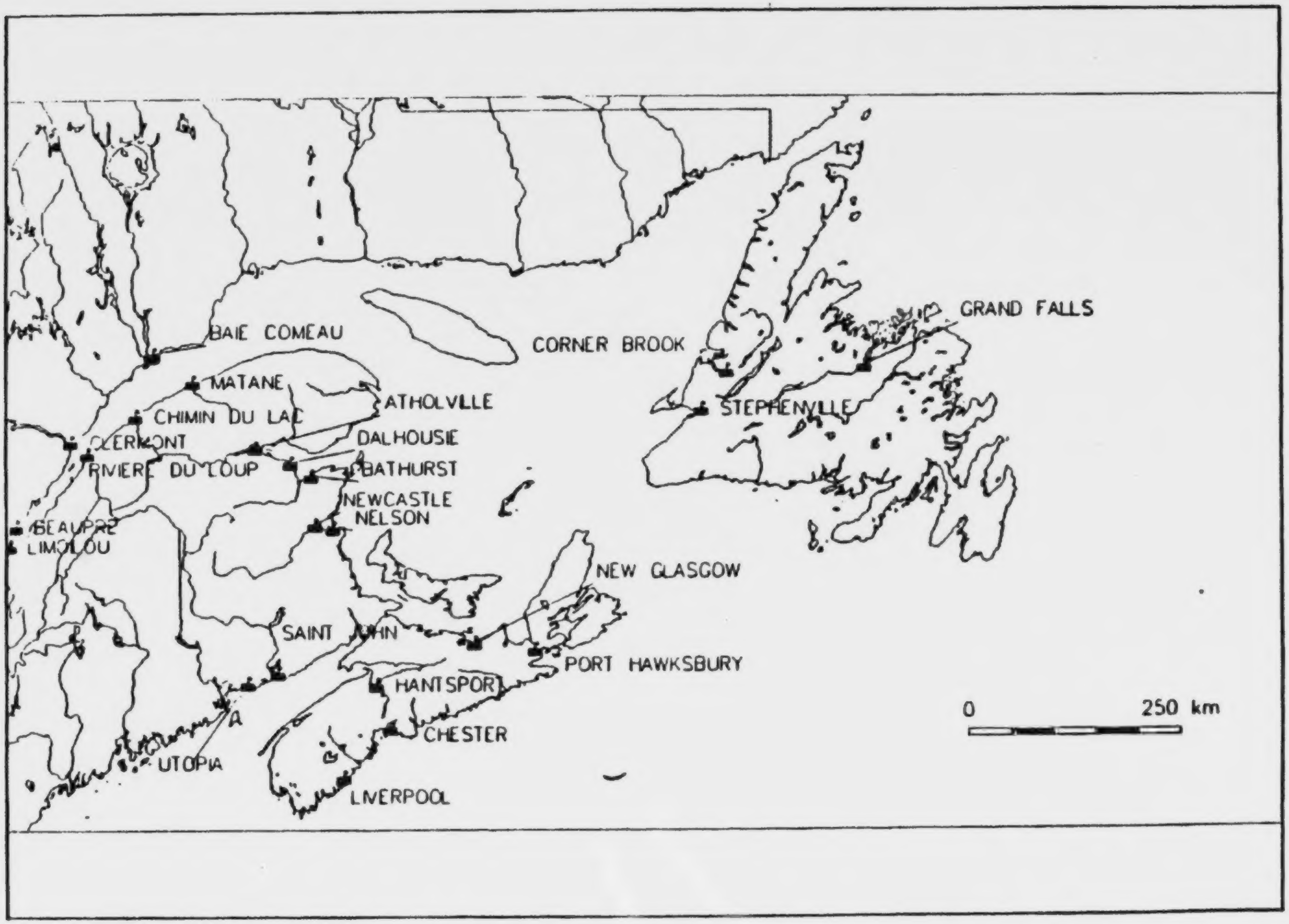

GRAND FALLS
BAIE COMEAU
CORNER BROOK
MATANE
ATHOLVILLE
STEPHENVILLE
CHIMIN DU LAC
DALHOUSIE
CLERMONT
BATHURST
RIVIERE DU LOUP
NEWCASTLE
NELSON
BEAUPRE
LIMOLOU
NEW GLASGOW
SAINT JOHN
PORT HAWKSBURY
HANTSPORT
0
250 km
CHESTER
A
UTOPIA
LIVERPOOL

FIGURE 2

LOCATIONS OF PULP AND PAPER MILLS IN THE FOUR ATLANTIC PROVINCES

BIOCHEMICAL OXYGEN DEMAND

BOD Loading

Pulp and paper mill effluent is a complex mixture of many substances including carbohydrates, lignins, organic acids and alcohols. Oxygen is consumed when bacteria degrades these materials. BOD is a measure of this oxygen demand. The majority of effluent oxygen demand can be met either in a mill treatment system, or if the effluent is not treated, the demand will be met by consumption of oxygen from receiving waters. Each day, ten B.C. mills discharge over 330 tonnes of oxygen demanding wastes to coastal waters in 2 million cubic metres of effluent (Table 1). Despite reductions of up to 56% between 1975 and 1980 at B.C. coastal mills (Kay 1989), the BOD loading from all but one pulp mill exceeds the BOD loading of municipal sewage from B.C.'s second largest coastal city, Victoria.

Sixteen mills in the Atlantic Provinces discharge 254 $t \cdot d^{-1}$ to estuarine and marine environments (Table 2). Total daily discharge loadings from Atlantic mills have been reduced 60% for BOD and 51% for TSS from 1969 to 1984 (Eaton et al. 1986). This has been achieved through a combination of process changes, in-plant controls and the addition of external treatment, mainly with aerated stabilization basins (Waldichuk 1988a).

Only one B.C. coastal mill has secondary treatment for about half its effluent, while eight Atlantic mills (six coastal, two inland) have secondary treatment.

Fate

The impact of mill effluent BOD on dissolved oxygen levels is moderated by the assimilative capacity of the receiving environment at each location. For example, at Elk Falls, B.C., where 52 $t \cdot d^{-1}$ of effluent BOD is rapidly mixed and dispersed by strong currents in Discovery Passage, there is little or no change in dissolved oxygen levels, even at the outfall. At the other extreme, where effluent is discharged into relatively quiescent coastal estuarine inlets which have restricted circulation (Port Alice, Port Alberni, Gold River, L'Etang Estuary), wide-scale, low dissolved oxygen levels are chronic and become serious during certain times of the year, especially when inlet flushing rates are reduced due to low river runoff. Unfortunately, this is also a time of fisheries sensitivity due to migrating salmon stocks.

It is important to note that some significant improvements have been made to BOD loadings by some mills. For example, prior to the installation of secondary treatment at the Fraser's Inc. mill in Edmunston, New Brunswick, and the Georgia-Pacific mill in Woodland, Maine, depressed levels of dissolved oxygen in the Saint John River and the St. Croix River, respectively, were observed. Since the implementation of secondary treatment at these mills significant increases in receiving water oxygen levels have been measured (Dafoe et al.1987, Eaton 1989). Similar improvements at Prince Rupert were documented when the sulfite process was replaced by kraft pulping (Kay 1989).

Biological Effects of Reduced Dissolved Oxygen

Mill effluent not only exerts an oxygen demand, it can reduce oxygen supply in receiving waters. Effluent colour can shade phytoplankton, thus reducing re-oxygenation via photosynthesis, and thereby reduce primary productivity in the estuary (Parker and Sibert 1972). This has been demonstrated near mills at Crofton, Port Alberni, Powell River (Anderson 1983) and at Port Mellon and Woodfibre (Stockner et al. 1975), and likely occurs elsewhere.

Lethal and sublethal responses of fish to decreases in dissolved oxygen include changes in behaviour, growth, swimming, respiration, fecundity, disease resistance, and feeding (Davis 1975, Birtwell 1989). These sublethal effects are more difficult to document in wild populations, but may be more widespread and significant than dramatic, serious events like fish kills, which may only rarely be documented. It also should be noted that the toxicity of kraft mill effluent increases when dissolved oxygen levels decrease (Marier 1973, Sprague 1985). Specific examples of effects related to BOD loading are given below.

Port Alice, British Columbia

Following recovery of sulfite liquor in 1977, the zone of serious BOD impact in Neroutsos Inlet was reduced from 20 km from the mill, to about 2 km (Kay 1989). However, during September and October 1985, the zone of potentially lethal (to salmon) dissolved oxygen concentrations (DO $<3 \text{ mg}\cdot\text{L}^{-1}$) extended over

5 km up and down-inlet from the pulp mill and included water depths from 0-20 m at some locations (Birtwell 1989). This recurring situation has persisted to October 1990 and is illustrated in Figure 3 for July 1987 (from Stucchi 1990). Measured dissolved oxygen levels fell below 1 mg·L^{-1} in 1985 (Colodey and Pomeroy 1986), and were thus inadequate for even some species of invertebrates (Miller et al. 1988). Non-salmonid mortalities, including herring and ratfish occurred (Colodey and Pomeroy 1986). Salmonid mortalities and stressed salmon were also demonstrated in 1985 using in situ juvenile chinook salmon bioassays (Kruzynski, DFO,pers. comm. in Birtwell 1989). Distribution of migrating salmon caught by DFO test-fishing nets was positively correlated to ambient dissolved oxygen concentrations (Colodey and Pomeroy 1986). The changes in maturation of these salmon were described by Birtwell (1989), who concluded that poor water quality blocked and hindered first-run salmon from entering their natal streams. He notes that late-run fish entered the creek to spawn during increasing dissolved oxygen levels in inlet waters and higher stream flows following strong winds and heavy rainfall.

In 1986 there were fourteen reported fish kills in Neroutsos Inlet (Colodey unpubl. file reports) ranging from 26 ratfish up to 10,000 dead herring, or on another occasion 90% of all intertidal life in a 10 km distance (pers. obs.). In five cases, mortalities are clearly related to low ambient dissolved oxygen (dissolved oxygen less than 1 mg·L^{-1}), while others may have been due to various factors such as effluent toxicity, or upwelling of hydrogen sulfide gas from the decomposition of deposited fibres and wood wastes . Stucchi (1990) reviewed the physical oceanography, dissolved oxygen and BOD loading in Neroutsos Inlet and concluded that a substantial reduction in the daily BOD loading is required

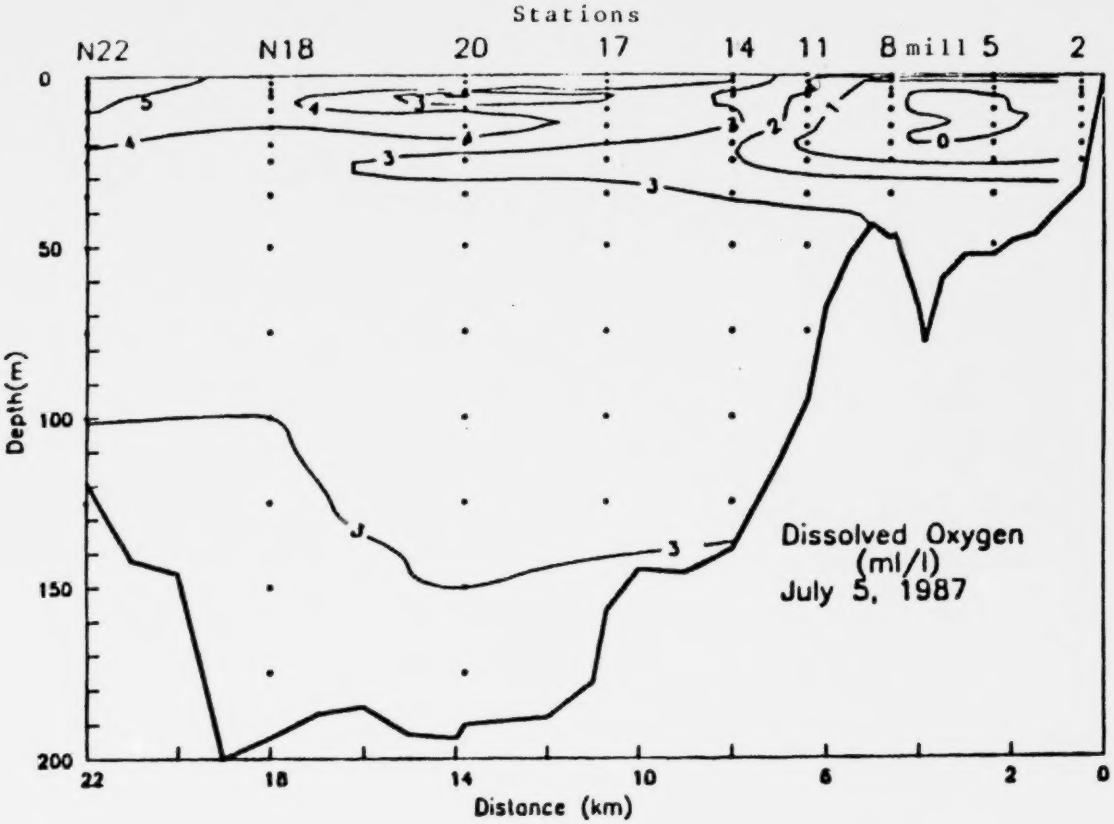


FIGURE 3 - AREA OF HYPOXIC WATER IN NEROUTSOS INLET, B.C.
(FROM STUCCHI 1990)

before Davis (1975) Level B dissolved oxygen levels can even be approached. (At Level B the average member of a species starts to exhibit symptoms of oxygen distress.) **Improved treatment facilities to achieve 80% reduction in BOD are** currently in the pilot stage of development at Port Alice.

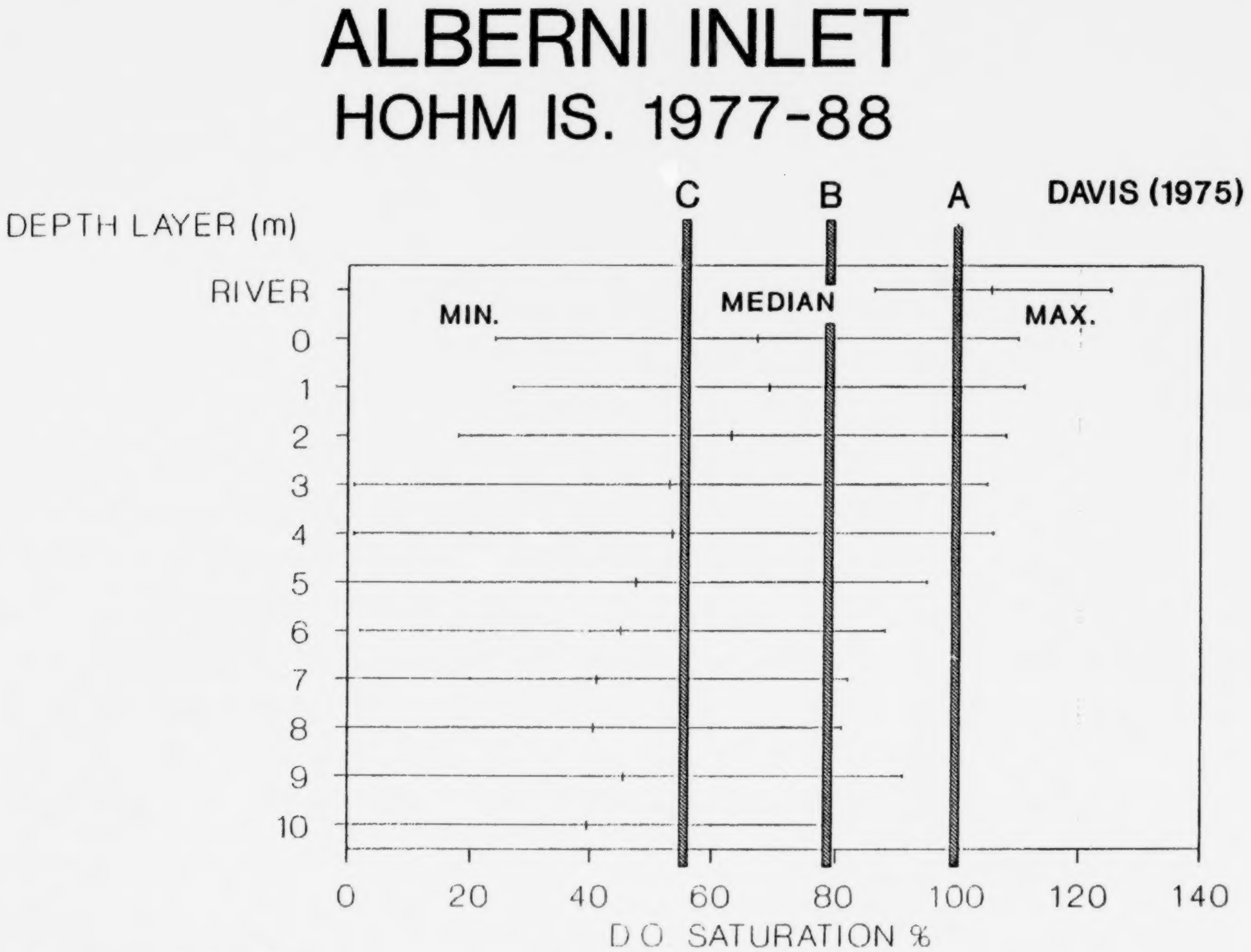
Port Alberni, British Columbia (see also section on TSS)

The situation at Port Alberni has been studied for many years since the modelling and BOD evaluation of the proposed sulfite mill by Tully in 1949. Waldichuk (1974) has reviewed the response of Alberni Inlet to increasing BOD loading as kraft mill production increased. He notes that there was no oxygen problem when the mill was producing 220 short tons per day of unbleached kraft pulp because of the low volume of waste. Dissolved oxygen problems were encountered when BOD loading rose in conjunction with increases in mill production. Although total production is now about 1300 tonnes per day, it should be noted that BOD loading has decreased from about 40 $t \cdot d^{-1}$ in 1970, to present averages of about 9 $t \cdot d^{-1}$, due to installation of an aerated stabilization basin in 1970 which treats about half the mill effluent. However, despite partial secondary treatment, weekly loadings of 14,500 $kg \cdot d^{-1}$ were exceeded on three occasions in 1985 (Dyer 1986).

In situ bioassays at Alberni Inlet have demonstrated that acutely lethal conditions occur due to low dissolved oxygen levels and to effluent toxicity (Birtwell and Harbo 1980, Birtwell and Kruzynski 1989). Use of the water column by juvenile and adult salmonids is restricted to the upper water layers, with highest effluent concentration and seasonally stressful temperatures, due to the low levels of oxygen in deeper waters. Recent studies on the impact of mill effluent (Colodey et al. 1988, Birch 1989, Stucchi et al. 1990)) have concluded that Alberni Inlet is receiving more oxygen demanding wastes than can be balanced by oxygenation processes, leading to a polluted situation with low ambient dissolved oxygen. Mill studies corroborated the observations of declining dissolved oxygen levels within the inlet (Hodgins 1989a). Davis' Level A criteria are not even met in the surface water layer for 50% of the data points collected from 1977-1988 as shown in Figure 4 (Colodey et al. 1988). Below 2 m median conditions were such that a large portion of the population would suffer a severe deleterious effect if exposed beyond a few hours (Davis 1975, Level C). Pre-mill data collected at Holm Island in 1941 (Pacific Oceanographic Group 1957) indicate that minimum dissolved oxygen saturation in the upper (0-2 m) and lower (4-10 m) water layers were much higher (88% and 20 % saturation respectively), than present minimum levels (20 % saturation in the upper and 1% saturation in lower layer). This demonstrates that the magnitude of the present very low dissolved oxygen conditions is not a "natural" occurrence of this inlet, although natural factors such as river flow and tidal exchange alter the degree of impact caused the combination of mill effluent BOD and TSS deposits within the harbour.

Gold River, British Columbia

Effluent from the bleached kraft mill at Gold River is discharged to Muchalat Inlet, which is very deep (> 300 m) and has restricted circulation. The dissolved oxygen minima in the upper 20 m water layer often coincides with effluent concentration maxima at each station sampled (Colodey unpubl. 1986, 1988 data). This lowered dissolved oxygen layer (1.5-3.0 $mg \cdot L^{-1}$) can extend up into



from Colodey et al 1988

FIGURE 4

FREQUENCY OF DISSOLVED OXYGEN VALUES BELOW LEVELS A, B AND C (DAVIS, 1975) IN ALBERNI INLET, B.C.

shallower depths (e.g. 2 m), thus lowering the amount of usable fish habitat within the inlet (Colodey unpubl. 1989 data). Oxygen conditions within the inlet could be further stressed with the increased BOD and TSS loading from the recent addition of a CTMP plant at the site.

L'Etang Estuary, New Brunswick

The Upper L'Etang estuary became incapable of supporting benthos and fish shortly after start-up of the semi-chemi-mechanical mill in 1970, due to the organic loading and resulting anoxia (Poole et al. 1978, Wildish 1983). Extensive descriptions exist of the major effects on the upper part of this small estuary, including the development of anoxic or hypoxic bottom waters, hydrogen sulfide production, the rapid elimination or gradual disappearance of indigenous species, and colonisation by hypoxic-tolerant benthic organisms. The benthic changes in the Lower L'Etang Estuary associated with mill discharges stabilized by 1972-1975 (Wildish et al. 1986), and water quality in the Lower L'Etang is adequate for aquacultural purposes as presently practiced. The multiple effects of aquaculture and effluent loading have resulted in some eutrophication, reduced diversity, and phytoplankton blooms and die-backs. Not to be forgotten are the long-term impacts of mill effluent on the bacteriological water quality, in terms of shellfish harvesting standards and contamination with *Klebsiella* spp. (Blaise and Legault 1973, Menon 1973). Consequently, the Lower L'Etang is closed to all bivalve shellfish harvesting.

Other Locations in British Columbia

Localised minor depressions in dissolved oxygen have been noted in the vicinity of effluent outfalls at Crofton (Colodey unpubl. 1989 data, Colodey and Tyers 1987, Sullivan 1980), Port Mellon (Cross 1989), and Woodfibre (Western Pulp 1988). No dissolved oxygen depressions have been noted near the outfalls at Harmac (Sullivan 1980, Colodey unpubl. 1989 & 1986 data), or Powell River (Sullivan 1980, Colodey unpubl. 1983 data). Dissolved oxygen levels at Porpoise Harbour have improved since the sulfite mill was shut down in 1976 and the diffuser brought on-line in 1978 for kraft mill effluent (Kay 1989).

Other Locations in the Atlantic Provinces

The Bathurst mill at the mouth of the Nepisiguit River, New Brunswick, reduces surface water dissolved oxygen concentrations when effluents are temporarily backed up by rising tides and low river flows. No significant oxygen depletion occurs when the tide is falling and effluents are flushed out into the harbour (I.E.C. Beak Consultants Ltd. 1985 a).

At Cornerbrook, Newfoundland, dissolved oxygen was reduced to 75% saturation up to 2 km from the Cornerbrook Pulp and Paper Limited mill located on the Humber Arm (Moores 1989).

TOTAL SUSPENDED SOLIDS

Loading

Suspended solids associated with pulp and paper mill effluent are largely composed of cellulose fibres, wood chips, and bark fines, although a smaller portion of the solids can be inorganic constituents such as boiler ash and calcium carbonate. The range of TSS loading for B.C. coastal mills is from 4 to 29 tonnes per day (Table 1). Atlantic mills have loadings ranging from <1 to 30 tonnes per day (Table 2). Mill efficiency with regard to fibre loss can be evaluated when TSS loading is divided by production. Values for B.C. coastal mills range from 7.7 to 17.7 kg TSS per tonne of pulp produced, whereas Atlantic region mills range from 3.3 to 39 kg TSS per tonne of pulp produced.

Fate

Waldichuk (1988b) recently summarised the effects of wood wastes on marine benthic organism and habitats. He noted that deposition of fibres can lead to conditions where benthic flora and fauna are totally eliminated. He concluded that wood fibres not only degrade benthic habitat but also lead to depletion of dissolved oxygen and production of toxic hydrogen sulfide. The long-term oxygen demand of mill effluent is related to its organic solids which form deposits on the seabed at many locations in British Columbia waters. At Port Alberni, for example, an elevation in sediment oxygen demand was demonstrated for degraded sediments which covered about 40% of the harbour bottom and accounted for about 10% to 55% of the total oxygen demand in the inner harbour (Hodgins 1989b, Stucchi pers. comm. DFO, Institute of Ocean Sciences, Sidney, BC). Hodgins states that: "... if the solids mat were to decrease in size through better retention and disposal of solids, then ultimately the benthic BOD would decrease. Studies reported in the literature indicate that this would not be a rapid recovery process."

Suspended solids loading causes the loss of productive benthic habitat when discharged solids settle and smother benthic organisms. A typical black, anaerobic deposit is often sampled near the outfall of most coastal mills. These deposits are often in the form of a jelly-like fibre mat which can be from several centimetres to 15 meters in depth (Pomeroy 1983). The release of methane gas, hydrogen sulphide gas (Waldichuk 1983, 1988b), and acids (Miller et al. 1979) along with organic contaminants are a serious threat to the receiving environment. The release of nutrients to the water column (Pearson 1982), although beneficial, is over-shadowed by benthic habitat degradation and oxygen demand, which has been measured to be as much as 3.6 g·m-2·˝d (Stein and Denison 1966, NCASI 1986). Figure 5A is a photo of a typical fibre mat area taken from the PISCES IV submersible, while Figure 5B shows the loss of benthic habitat by coarse wood material from log-booming operations at Port Alberni. Sediment oxygen demand under log-booming areas in the Coos River Estuary and areas with bark and wood debris in Alaska have been measured to be 2 - 4 g·m-2·˝d (Schaumberg 1973, Jones & Stokes Inc. 1989).

Present zones of measurable impact range from about 0.5 km to 5.0 km from the mills, covering several km^2 at most mills. For example, benthic degradation at

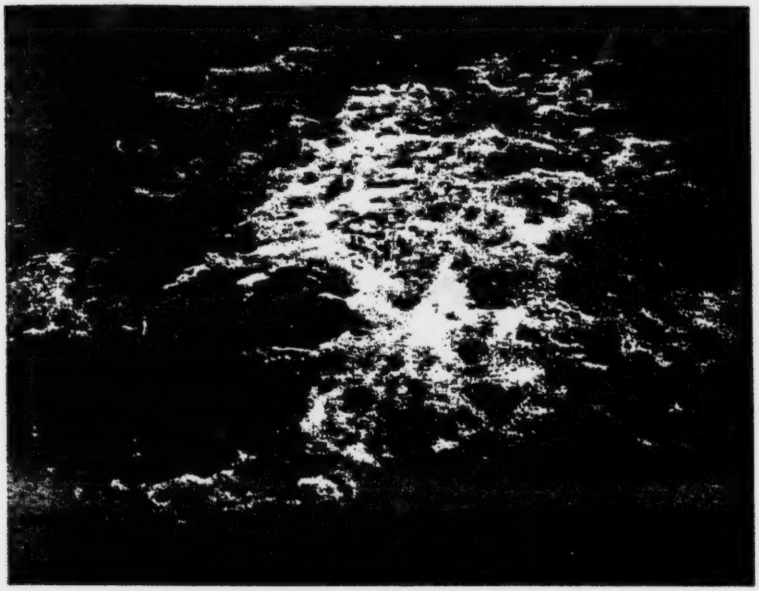


PLATE 1 Appearance of typical fibre deposit - Northumberland Channel off Harmac pulpmill

FIGURE 5a PLATE 1. APPEARANCE OF TYPICAL FIBRE DEPOSIT - NORTHUMBERLAND CHANNEL OFF HARMAC PULPMILL

PLATE 2 Wood debris and development of white bacterial slime found under sorting and storage areas - Alberni Inlet in vicinity of Port Alberni pulpmill

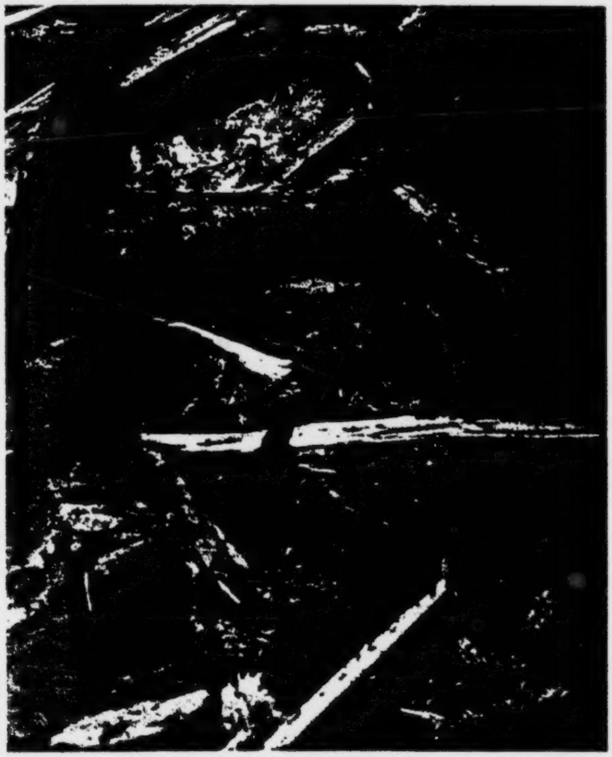


FIGURE 5b PLATE 2. WOOD DEBRIS AND DEVELOPMENT OF WHITE BACTERIAL SLIME FOUND UNDER SORTING AND STORAGE AREAS - ALBERNI INLET IN VICINITY OF PORT ALBERNI PULPMILL

B.C. coastal mills ranges from 1 to 8 km^2. Insufficient data are available to determine the rate at which solids deposition is encroaching on the benthic habitat, although it has been suggested that at some locations mats are in equilibrium (e.g. Harmac: Ellis and Ostrovsky 1983). In one instance where fibre accumulations have been adequately sampled, we can conclude that their size is relatively stable and fluctuates from year to year (e.g. Crofton: Colodey and Tyers 1987).

In the Atlantic Provinces, the results of a monitoring program on the Humber Arm, Newfoundland, indicated that the major impact from the Cornerbrook mill effluents was the smothering of benthos by wood fibre for a distance of 2 km north and northeast of the point of discharge (LeDrew and Bennett 1989, Moores 1989). This mill had the highest fibre loss per tonne of pulp produced (39 kg), when Canadian coastal mills are considered. The active deposition in the Arm of coarse wood material, from log drives and debarking, is now stopped (LeDrew and Bennett 1989).

Similarly, sediment cores taken in Liverpool Harbour, Nova Scotia, indicated that Organic Sediment Index values were elevated at stations up to 1 km from the outfall of the Bowater Mersey mill (Beak 1971). A major benthic deposit of wood fibre has been present off the Stora Mill at Point Tupper, Cape Breton, for at least twenty years (Machell et al. 1978).

It is expected that effects on benthos, even after TSS deposition stops, will occur over many decades in areas with low natural sedimentation rates.

Biological Effects of Effluent Solids

Resource species, as well as smaller invertebrates used as food sources, may be replaced by fewer kinds of less desirable, pollution-tolerant species when organic deposits change the characteristics of the sediment. Changes to invertebrate species diversity and abundance in response to increasing sediment organic content have been described for Porpoise Harbour, Port Alberni, Port Mellon, Port Alice, Harmac and Ocean Falls by McGreer (1984). Degraded sediment from Port Alberni and Port Mellon are toxic to amphipod crustaceans in laboratory bioassays (Chapman and Barlow 1984). Fish kills have been caused by upwelling of toxic gases from benthic deposits at Powell River (B. Moore, pers. comm. BCMOE, Surrey, BC). Even at Elk Falls, where strong currents disperse the effluent, there are degraded sediments (wood chips and indication of pulp). Near the outfall, benthic species response is reduced abundance and diversity in comparison to the reference location (Morrow Engineering 1985).

The recovery rate following cessation or reduction of solids deposition is a function of site-specific sedimentation rates and larval supply, both of which are mediated by local current regimes. It may be decades until natural sediments cover the historical inputs (up to 15 m thick) which have accumulated at many sites, such as at Ocean Falls in British Columbia (Pomeroy 1983, Fournier and Levings 1982).

Reduction in TSS loading, through improved levels of effluent treatment is required to prevent further habitat degradation and begin the recovery period as quickly as possible. Since a large portion of hydrophobic organic chemicals (like dioxins) are associated with suspended solids (Muir et al. 1985, Servos and Muir 1989), solids control could also be expected to limit the release of particulate associated contaminants. Settling suspended particulate matter (SPM) is of great importance for the introduction of these contaminants to a variety of organisms, where uptake is mainly by ingestion of SPM (Broman et al. 1989).

TOXICITY: ACUTE LETHALITY

Loading

Acute bioassay tests are used in accordance with federal regulations to evaluate the lethality of the mixture of toxic compounds found in mill effluents.

One review (Sprague and Colodey 1989, using data from McLeay 1987) calculated that untreated kraft mill effluent had a 96 h LC50 of 16% (killed 50% of the test fish when exposed to 16% effluent for 96 h). Only one B.C. coastal mill has secondary treatment for about half its effluent, and as a result B.C. marine discharge mills in toto have rather toxic effluents (median LC50 = 32%, Table 1), in relation to B.C. freshwater discharge mills where secondary treatment is required (all LC50 = 100%). On the east coast, mills have final effluent toxicities ranging from 96 h LC50's of 2% to >100% (Table 2). It can be seen that one east coast mill accounts for 50% of the region's toxic effluent emmissions (as expressed as TER in Table 2).

In the early 1980's, three mills in northern New Brunswick were studied extensively. Consolidated-Bathurst effluent samples, mixed from four sewers in proportion to flow, were found to have 96 h LC50's to Atlantic salmon parr of 32-56%, 10-32% and 0-10%. Miramichi Pulp and Paper effluent samples had LC50's of 56-100% and greater than 100%, while effluent samples from Atholville Pulp had LC50's of 10-18% and 10-32% (Boudreau et al. 1988).

Well-designed and operated secondary treatment facilities are an effective way to eliminate effluent toxicity of kraft mill effluent, largely by the decomposition of resin and fatty acids (Sprague and Colodey 1989). Secondary treatment (especially activated sludge) can also be expected to reduce the loading of some chlorinated organic compounds (Gergov et al. 1988, Fleming et al. 1990, *inter alios*).

Biological Effects of Effluent Toxicity

The aquatic toxicity of pulp and paper mill effluents has been extensively reviewed by McLeay (1987) and Sprague and Colodey (1989). Recently published studies described in situ field bioassays at Port Alberni and Port Mellon, British Columbia which demonstrated toxicity of partially treated and untreated BKME to several species of salmon (Birtwell and Kruzynski 1989). The reader is also referred to the section dealing with organochlorines for more information on effluent toxicity, particularly as it relates to organochlorine substances and sublethal effects.

Even when effluent passes the acute bioassay test (at least 50% of the fish survive) and is deemed "non-acutely toxic", it is possible that it can cause other detrimental effects. Examples from the freshwater environment illustrate the point. A study near the Proctor and Gamble bleached kraft mill at Grand Prairie (Alberta), which discharged treated non-toxic effluent, showed that downstream fish had accumulated chlorinated effluent compounds and had liver damage when compared to upstream fish (AEC 1987). Histological liver damage has been demonstrated in marine and freshwater fish exposed to BKME (Brand and Goyette 1990, Smith et al. 1990). A recent study by Munkittrick et al.(1989, 1990) demonstrated that fish exposed to bleached kraft mill effluent had elevated liver enzyme activity, coupled with reduced steroid hormone levels. These metabolic changes were associated with reduced sexual development in male and female whitefish. In another instance, plasma testosterone levels in trout were decreased by exposure to BKME in a lab study (Lindstrom-Seppa and Oikari 1989a). Liver enzyme activation has also been demonstrated in fish exposed to BKME on the Fraser River (Servizi et al. 1990), Thunder Bay (Smith and Rokosh 1989, Smith et al. 1990), St. Maurice River (Hodson et al. 1990), and Finland (Lindstrom-Seppa and Oikari 1989b, 1990). These preceding studies confirm results described in Swedish work (Andersson et al. 1988).

The above discussions on toxicity have focused primarily on the toxicity of whole effluents to fish. However, all ecosystem components must be protected from effluent toxicity. In this regard, Anderson (1983) previously demonstrated that copepods, which are an important part of the food web, are more sensitive to kraft mill effluent (LC50 = 12%) than juvenile salmonids (LC50 = 25%). The toxic effect of bleached kraft mill effluent constituents has also been demonstrated using sea urchins (Cherr et al. 1989) and copepods (Renberg et al. 1980).

Pulp mill effluent chlorate concentrations rise when chlorine dioxide is substituted for chlorine gas (Munro et al. 1990). Effluent chlorate has been shown to be toxic to brown algae (*Fucus vesiculosus*) at 10-20 ug·L^{-1} when ambient nitrate levels were low (Lehtinen et al. 1988). It should be noted that several chlorate spills (up to 45% concentration) have occurred during the past few years at Pacific coast marine pulp mills during loading or unloading operations (EP Spills Database). As mills increase their use of chlorate for chlorine dioxide generation, more care will have to be used to limit its entry into the aquatic ecosystem, through proper handling and treatment system design. Chlorine dioxide, itself, is two to four times more toxic than total residual chlorine to freshwater fish with a 96 h LC50 of 20 ug·L^{-1} for juvenile fathead minnows (Wilde et al. 1983). However, effects of chlorine dioxide on marine organisms occur only at relatively high concentrations: kelp germination and sea urchin malformations at 250 mg·L-1 (Hose et al. 1989).

Wu and Levings (1980) demonstrated that blue mussels and barnacles suffered reproductive impairment and slower growth rates when transplanted near the Port Mellon kraft mill outfall. The authors note that the results of their study correlated well with a previous study at the location which found low densities of barnacles and mussels near the outfall (Levings and McDaniel 1976).

In the Atlantic Provinces, three mills in New Brunswick (Bathurst, Newcastle, Atholville) created extensive effluent plumes in the receiving estuaries. During rising tides, the Nepisiguit River had concentrations ranging from 1 to 10% effluent across most of its width and greater concentrations near the sewers. Under similar tidal conditions, the Miramichi River had 1 % effluent across its entire width. The Restigouche River, however, had areas on its north side where effluent could not be detected at any part of the tidal cycle. Behavioural studies in the laboratory with Atlantic salmon parr showed that effluents from the three mills at high concentrations (10% and 100%) increased their locomotor activity. Behavioural responses (changes in activity) occurred at 1% effluent concentrations for all mills, and concern was expressed that salmon migration might be affected in the receiving estuaries (Boudreau et al. 1988). In a follow-up 1985 at the Consolidated-Bathurst mill in Bathurst, New Brunswick, it was shown that juvenile Atlantic Salmon would avoid mill effluent at a concentration of 3.5%. Under low flow river conditions, a plug of effluent at, or above that concentration was predicted to form in the freshwater layer at hightide and to flow downstream until low tide was reached (I.E.C. Beak 1985 b).

IMPACT OF ORGANOCHLORINE SUBSTANCES

Loading

The impacts and toxicity of pulp mill related organochlorine substances have been recently reviewed (Colodey 1989, Sprague and Colodey 1989). About 200 low molecular weight chlorinated compounds have been identified in kraft bleach plant wastes (Reeve and Earl 1988, Suntio et al. 1988, McKague et al. 1989). This represents only a portion of the total number of chlorinated compounds represented as AOX (Adsorbable Organohalogens). A single large kraft mill may discharge around 4 tonnes of organically bound chlorine or about 50 t/d chlorinated organic substances (Sprague and Colodey 1989). AOX data for B.C. coastal mills (Table 3) indicates organically bound chlorine loadings of 1-26 t/d, compared to limited data from Atlantic Region mills which indicate that they discharge from 1-5 t/d (Table 2). The untreated effluents from these mills have the potential for introducing over 33,000 tonnes of organically bound chlorine into the receiving waters annually. This could represent over 400,000 tonnes of chlorinated organic compounds, assuming a conversion factor of thirteen from organically bound chlorine (AOX) to chlorinated organic compounds (Sprague and Colodey 1989).

Fate

Once discharged into the environment, these compounds can become sorbed to particles (Broman et al. 1989, Servos and Muir 1989), dispersed and contaminate a large area. Chlorinated organics from pulp mills on the Fraser River were detected in water and fish in the Fraser Estuary up to 750 km downstream (Carey and Hart 1988, Rogers et al. 1988a, Rogers et al. 1988b, Servizi et al. 1988). AOX from pulpmills persisted over 1250 km in the Athabaska River during winter sampling (Noton 1990). The linear dispersion distances of dioxins, chloroguaiacols and chlorocatecols from some B.C. coastal mills is shown in Table 4. Just as dispersion distance depends on site-specific variables, recovery of contaminated sediments by burial with clean sediment will also be

TABLE 3 - AOX DATA FOR BC COASTAL MILLS

Mill	AOX (kg·adt^{-1})[1]	Loading (t/d)2
Crofton	4.4-7.1	8.4-13.7
Elk Falls	6.0	12.2
Port Mellon	5.8	3.8
Harmac	5.5-7.1	5.7-7.4
Port Alberni	5.8	7.5
Powell River	9.0-14.9	15.8-26.1
Prince Rupert	0.5-3.1	0.5-3.5
Port Alice	5.2	2.4
Woodfibre	3.7	2.3
Gold River	6.2	4.3

TOTAL 62.9-83.2

1 CPPA Data, Sept. 15, 1989

2 1987 Production Data

TABLE 4

Distances (km) from mill outfalls where trichloroguaiacol (TCG), tetrachloroguaiacol (TeCG), trichlorocatecol (TCC), tetrachlorocatecol (TeCC), and 2,3,7,8-tetrachlorodibenzo-dioxin (2,3,7,8-TCDD) have been detected in sediment and biota (data from Dwernychuk 1989)[1].

Location	TCG	TeCG	TCC	TeCC	2,3,7,8-TCDD			
	Sediment		Sediment		Sediment	Crab	Shrimp	Mussels
Prince Rupert	>6	>6	>6	>6	>6	>6	2	<1
Woodfibre	5	10	8	10	4	>16	<1	<1
Port Mellon	6	8	5	>10	>10	>22	1	<1

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[1] ">" means the contaminant was found at the furthest distance sampled.

related to local sedimentation and erosion rates, and the degree to which the sediments are modified by biological communities (bioturbation). It has been demonstrated that levels of 2,3,7,8-TCDD (dioxin) in contaminated sediment from Newark Bay, N.J., have declined considerably over the past 25 years. However, some areas with low net sediment accumulation and active sediment mixing have the highest contaminant concentration in surficial sediments (Bopp et al. 1988). These sediments would therefore be available as a biological contact surface.

Other studies predict that food-chain uptake is the main bioaccumulation route for polychlorinated dibenzo-p-dioxins in aquatic environments, especially when water concentrations are low (Servos and Muir 1988, Muir and Yarechewski 1988, Muir et al. 1988). Such food-chain models are recommended as the preferred method for calculating site-specific dioxin body burdens (Dudley and Wagner 1989). Although 2,3,7,8-TCDD fate has long been studied in freshwater model ecosystems (Isensee and Jones 1975, Corbet et al. 1983, *inter alios*), results are not available for marine and estuarine systems. Freshwater studies indicate that bioaccumulation of 2,3,7,8-TCDD occurred primarily through the food chain and secondarily through contact with contaminated sediment, when rainbow trout were exposed in the laboratory. The water-exposure route did not appear to make a significant contribution in this case (Batterman et al. 1989).

The environmental fate and biological effects of these compounds is further complicated because some higher molecular weight compounds (e.g. chloroguaiacols) can be degraded and transformed into other more toxic compounds (e.g. chloroveratroles: Neilson et al. 1984, Neilson 1989, Paasivirta 1987). Chloroveratroles and chloroanisoles bioaccumulate and have been linked to tainting in some species of fish (Paasivirta 1988). The tainting potential of 2,4-dichlorophenol is reflected in the 0.2 ug·L^{-1} guideline set in the Canadian Water Quality Guidelines (CCREM 1987). This compound becomes a concern given the shift to lower chlorinated compounds during ClO_2 substitution. This tainting potention is not reflected in the proposed pentachlorophenol toxic equivalent units (TEU: Flemming et al. 1990). For example, the final effluent levels of 2,4-dichlorophenol at the Terrace Bay mill in Ontario would require 100-fold dilution in order to meet the CCREM guidelines (07/24/89 sampling date- DOE database).

A study of the fate of organochlorine compounds from the 750 ton·d^{-1} bleached kraft mill at Saint John, NB (Bacon 1978, Bacon 1980, Bacon 1983) showed the presence of dichlorophenols, trichlorophenols, trichloroguaiacols and tetrachloroguaiacols in sediments, clam (*Mya arenaria*) extracts, tomcod and flounder liver, and confirmed the earlier results of Brownlee and Strachan 1977. Pentachlorophenol was markedly present in all animals examined, and may have been related to the use of wood preservatives. A wide range of concentrations (not given) were detected, all at presumably sublethal levels. Flounder, smelt and tomcod accumulated the compounds, in liver and fat (flounder), viscera (tomcod) and liver (smelt), respectively. Resin acids and fatty acids were only detected in clams and tomcod from one location.

Biological Effects of Effluent Organochlorine Compounds

The biological effects of chlorinated compounds found in pulp and paper effluents on marine and estuarine biota are not well known. Lab studies on the uptake and depuration of chlorinated phenols and guaiacols in mussels agreed with field results which indicated the presence of these contaminants in biota from Saint John Harbour and the Northumberland Strait near New Glasgow. The uptake and depuration of these compounds were also studied using a small estuarine fish (*Fundulus heteroclitus*). Histological damage was demonstrated when fish were exposed to either 0.5 mg·L^{-1} trichlorophenol (TCP), or trichloroguaiacol (TCG) or 2.5 % bleachery effluent. Liver damage was only completely reversible in fish exposed to TCP and TCG, but not in fish exposed to 2.5 % bleachery effluent (Bacon 1980), suggesting that other compounds may also be involved.

Artificial stream studies using well-treated (14 d aerated lagoon) bleached kraft mill effluent demonstrated statistically significant lower survival in 4 of 5 effluent tests using rainbow trout (NCASI 1989 p. 97; cf. Flemming et al. 1990). Natural colonization of streams by Mountain Whitefish was also much lower in stream channels receiving effluent (n=8,9) compared to control streams (n=58,20; NCASI 1989 p. 122). Enhanced growth marocinvertebrates (due to nutrient enrichment) and of exposed trout (due to lower densities) were described by Hall et al. 1990. It is estimated that treatment streams were receiving effluents having AOX (adsorbable organohalide) levels of 3 to 4 kg·t^{-1} (Flemming et al. 1990).

Recent studies on Fraser River juvenile chinook salmon exposed to treated BKME support the importance of the food chain uptake of dioxin, demonstrate liver enzyme activation (EROD induction 2.5 times control levels) and tentatively identify liver granulomas, as healing bacterial kidney disease

(Servizi et al. 1990). Fish growth and seawater acclimation were not affected by effluent exposure. Lab exposure of fish to treated BKME led to contaminant uptake (chlorophenols, chloroguaiacols, EOCL= extractable organochlorine) in proportion to effluent exposure. Field studies (Rogers et al. 1989) indicated similar levels of organochlorine contamination in feral fish, but much higher levels of EROD induction (55 times control).

The effect of BKME on fish in the St. Maurice River (Quebec) show a similar pattern of response to effluent exposure as Fraser River fish (Hodson et al. 1990). Liver AHH enzyme activity was elevated in exposed white suckers 5-10 fold at sites up to 95 km downstream. Changes in enzyme induction match changes in liver somatic index (LSI), hematocrit, serum glucose, serum protein and fin-ray asymmetry, and were all clearly related to effluent exposure. A decrease in gonad somatic index (GSI), for both sexes at the 95 km site indicate that sexual maturation was retarded, a suggestion supported by changes in serum hormone levels measured for both sexes. Too few northern pike were sampled to be conclusive, but they seem to demonstrate a similar pattern for AHH induction and LSI as white suckers. Similar reproductive changes, either to organs or hormone levels were described by Munkittrick (1989), and Lindstrom-Seppa and Oikari (1989a). Andersson et al.1988 and Sandstrom et al. 1988 respectively, found gradients in liver enzyme activity and reduced gonad size and development at a Swedish bleached kraft mill.

Some data is available on marine species. One study has examined the effects of blue crabs consuming radio-labelled 2,3,7,8-TCDD (dioxin) contaminated clams (Cristini et al. 1989). It was determined that the digestive gland (hepatopancreas) of the crab accumulated the highest concentrations of TCDD, which is consistent with data from B.C. organochlorine surveys (Dwernychuk 1988a, 1988b, Whittle et al. 1990). Some 2,3,7,8-TCDD was detected in the fecal material which the authors interpreted as excretion, but may be a result of incomplete assimilation. In a separate experiment, crab were fed dioxin and furan contaminated (137 ppt) clams from Newark, N.J. (Cristini et al. 1989). The physiology of these crabs was disrupted in that they had fewer molts and slower limb regeneration compared to crabs from the control area.

A clam transplant study (Cristini and Cooper 1988) was used to evaluate the bioavailability and physiological effect of dioxins and furans on the clam (*Mya arenaria*). Exposed clams (at Elizabeth and Newark, NJ) accumulated dioxins and furans, had reduced length, width, and shell-meat ratios, exhibited significant lesions in the digestive tract and hepatopancreas, and had lowered adenylate energy charge. The authors concluded that the environmental exposure to dioxins and furans could alter physiological processes. A later study demonstrated shell-thinning and a variety of lesions of the gill, kidney and digestive gland. (Cooper et al. 1989).

The fecundity of crustacean copepods was reduced when they were exposed to 37-54 $ug \cdot L^{-1}$ tetrachloroguaiacol, whereas the 96 h LC50 of this compound was a thousand-fold higher (39 $mg \cdot L^{-1}$; Renberg et al. 1980).

The subtle nature of the mode of action of some chlorinated compounds is perhaps best illustrated by the delayed mortality to rainbow trout fry exposed to 38 part per quadrillion 2,3,7,8-TCDD: fish died after a 28 day depuration phase which followed the 28 day exposure phase (Mehrle et al. 1988). Other examples of delayed mortality in fish exposed to 2,3,7,8-TCDD are cited by Muir and Yarechewski (1988).

The bioaccumulation of some of these compounds (dioxins and furans) to levels higher than public health standards has resulted in the restrictive use or closure of over 67,000 hectares of productive fisheries and wildlife habitat in British Columbia. Although half-lives for some dioxin and furan congeners have been calculated for some species of freshwater fish like fathead minnows, trout (Niimi and Oliver 1986, Muir and Yarechewski 1988, Muir et al. 1989), carp (Kuehl et al. 1987) and yellow perch (Kleeman et al. 1986), similar data for marine species of fish and invertebrates are unavailable. It is known that invertebrates accumulate dioxins and furans in marine environments with little or no selectivity due to metabolism, and that fish can metabolize non-2,3,7,8 congeners greater than 2,3,7,8 congeners (Norstrom and MacDonald 1989).

The impact of chlorinated organic compounds like dioxins are not limited to aquatic organisms. Great Blue Herons (*Ardea herodias*) have been shown to be a valuable indicator of organic contaminants, like PCB, HCB, DDE, TCDD and TCDF in British Columbia. (Whitehead 1989). Concern was raised about the possibility that TCDD was implicated in the failure to fledge young at the Crofton heron colony in 1987 (Elliott et al. 1989). A laboratory study has since shown that eggs contaminated with higher levels of dioxins exhibited reduced growth, edema, liver enzyme (EROD) activation and other morphological and physiological changes (Hart et al. 1990, Bellward et al. 1990).

The prudent approach is to limit the entry of chlorinated compounds into the environment by instituting effluent controls on the chlorinated compounds as a class of substances and by minimizing TSS loadings, since the fate and biological effects of these compounds are largely unknown.

CONCLUSIONS & RECOMMENDATIONS

Smith et al. 1990, Hodson et al.1990, Munkittrick et al. 1990 and Sprague 1990 concluded that recent Canadian studies are consistent with Scandinavian results on the effects of BKME. Owens 1990 points out that some of the Scandinavian results are related to conventional parameters (BOD & TSS) and recommends an integrated approach to future studies which would combine field, laboratory, chemical, toxicological and sublethal approaches.

Studies are needed to quantify the responses and describe the health of fish exposed to BKME in the marine and estuarine environments, using current diagnostic health and ecotoxicological techniques. The sublethal effects of effluents and organochlorines can be partly assessed by new laboratory tests and environmental effects monitoring protocols proposed under the changes to the federal regulations. Hazard assessments and environmental effects monitoring programs at each mill site are needed to complete the accurate assessment of potential sublethal effects and bioaccumulation dynamics of organochlorine compounds on a site-specific basis. There is a clear need to eliminate acute toxicity of effluents, especially in situations where the effluent is discharged to areas of shallow water or constricted areas through which migrating fish must pass. These changes are expected when the revised Pulp and Paper regulations become implemented.

In conjunction with field biomonitoring and hazard assessments, long-term bioassays are needed for assessing the effects of specific chlorinated compounds alone and in combination with pulp and paper effluents under environmentally realistic conditions of dissolved oxygen and temperature. Recent work (Randall 1990) indicates that hypoxic conditions may alter contaminant uptake dynamics.

As chlorine dioxide substitution rates increase, there is a need to track Canadian mill effluent chlorate levels. The release of chlorate into the environment should be minimized by treatment system design and through proper chemical handling procedures at mill sites. There is a concurrent need to evaluate chlorate toxicity with local algal species as well as with standard test species.

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PLATFORM SESSION

Marine Environmental Quality Monitoring

Chairs: M. Pomeroy and M. Waldichuk

MONITORING ORGANIC CONTAMINANTS IN CANADIAN SEABIRDS, 1968-1990.

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Since 1968, eggs of selected seabird species have been collected at four-year intervals from colonies in eastern Canada and analyzed for organochlorines. Periodic surveys of organochlorines in seabird eggs have also been conducted at colonies on the Pacific coast and in the arctic. This monitoring program was established to provide data on contamination of the marine environment and possible implications for seabird health. DDE and PCBs declined significantly in all species from both the Bay of Fundy and the Strait of Georgia by the early 1980s, but levels now appear to have stabilized. Generally DDE has declined more than PCBs. Dieldrin, oxychlordane, HCH and mirex levels have declined at some locations but were stable at others. HCB and heptachlor epoxide levels remained steady or increased depending on the species and location. All measured organochlorines increased or remained steady between the mid-1970s and mid-1980s in a resident arctic seabird, the ivory gull (Pagophila eburnea). GC/MS analysis of Leach's storm petrel eggs from Newfoundland in 1964 showed that toxaphene levels were greater than PCB levels, and had increased about two-fold over levels in eggs collected in 1968 and archived in a specimen bank.

LINKING ENVIRONMENTAL INFORMATION TO DECISION-MAKING: THE ROLE OF MONITORING, REFERENCE THRESHOLDS AND INDICATORS

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ABSTRACT

To manage ecosystems and their resources on a sustainable basis, sound information must be available on which decisions can be based and evaluated. Environmental reporting -- the timely delivery of understandable environmental information -- is an important tool which can help provide this information to decision-makers and the public. In recognition of this, Environment Canada is leading a federal effort to develop a national profile of environmental indicators.

This paper examines how environmental indicators can be used to focus environmental information and facilitate its use for decision-making and public communication. The indicator concept is introduced, and the basic building blocks required for indicator development -- such as environmental monitoring and reference thresholds -- are discussed. Examples of indicators which have been used successfully are presented and analysed. The ability of existing data sets to allow for the use and development of marine environment indicators is reviewed and found wanting. Suggestions are made from an environmental indicator viewpoint regarding the development of a national marine monitoring program. The need for data which permit temporal and spatial analysis at national and regional scales is emphasized.

Paper presented at the Marine Environmental Quality Monitoring Platform Session of the 17th Annual Aquatic Toxicity Workshop, November 5-7, 1990, Vancouver, Canada.

1. INTRODUCTION -- THE NEED FOR ENVIRONMENTAL INFORMATION

We live in a period of heightened awareness and concern about the state of the environment. The issues which are fuelling public and scientific concern range from those which are global in nature with the potential to affect all of humanity (such as stratospheric ozone depletion) to those which are regional or of importance in a specific location (such as poor water quality). This concern will intensify as human populations and environmental pressures expand and as more is learned about the nature and significance of environmental problems.

In Canada, as in many other countries, the public is demanding more information about the state of the environment, about how it is changing over time, and about the ecological, economic and health-related significance of current conditions and trends. Equally important is the demand for actions to resolve existing environmental problems and for strategies which anticipate and prevent potential and emerging problems.

These demands are increasing pressures on decision-makers at all levels. In 1987, the World Commission on Environment and Development served urgent notice that "based on the latest and best scientific evidence, decisions must be taken now to ensure sustainable human progress and human survival" (World Commission on Environment and Development, 1987). Canadian marine managers are under pressure as illustrated by present concerns regarding, for example, the state of the fishery and the presence of highly toxic dioxin and furan contaminants in shellfish, seabirds and marine mammals.

It goes without saying that sound decisions require sound information. Canadians as decision-makers want to receive information about the state of the environment in the same systematic way they receive information about the state of the economy (Government of Canada, 1990). In the marine environment, intelligent decisions regarding the use and allocation of resources require consistent information about the status and trend of environmental quality in coastal areas. Key questions include whether environmental conditions are stable, improving or deteriorating, how conditions in different areas compare with one another and which specific areas require special attention, research and remedial action.

Environmental reporting -- the timely delivery of understandable environmental information -- is an important tool which can provide environmental information to decision-makers and the public. This paper will examine how environmental reporting, through the use of indicators, can facilitate this process. The indicator concept will be introduced and the basic building blocks required for indicator development -- such as environmental monitoring and reference thresholds -- will be reviewed. Examples of currently used environmental indicators will be presented and their essential features explained. Finally, the ability of current marine monitoring programs to meet environmental reporting needs will be examined and suggestions made regarding the collection of additional information.

2. DEFINING AND CHARACTERISING ENVIRONMENTAL INDICATORS.

Over the past twenty years (ie. since the Stockholm Conference) governments have increased the resources aimed at environmental protection and conservation. The growth of non-government action has been at least as significant. However, many unanswered questions remain. One important group of questions relates to how we will know whether or not progress is being made. Economic indicators such as the Gross National Product, the Consumer Price Index and the national current account reveal key information about economic performance. In contrast, there are few systematically reported indicators which inform us of trends in the state of the environment and on which past management decisions can be evaluated.

Interest in sustainable development and growing public concern about environmental pressures have stimulated governments to re-examine their capacity to assess and monitor the state of the environment and detect changing conditions and trends. Pressures are also growing for measures of performance, thus the subject of environmental indicators has come to attract considerable attention as a necessary tool for helping to chart progress being made toward a sustainable future (Organisation for Economic Cooperation and Development, 1990).

This interest is reflected in several recent initiatives. At the international level, in May 1989 the OECD Council meeting at Ministerial Level called, inter alia, for a next generation work programme on environmental economics that would integrate environment and economic decision-making more systematically and effectively as a means of contributing to sustainable development. In July 1989 the Paris G-7 Economic Summit (the annual summit meeting of the world's seven leading industrial democracies) reinforced this and called on the OECD "within the context of its work on integrating environment and economic decision-making, to examine how selected environmental indicators could be developed". Here in Canada, Environment Canada is leading a federal effort to develop a national prototype set of environmental indicators based on existing data.

2.1 Indicators Defined. A review of the literature reveals that the term "indicator" has achieved widespread use in many disciplines, including environmental reporting. Perhaps due to this widespread use, the term's meaning appears to have expanded to the point where it is commonly used as a synonym for data (Gelinas and Slaats, 1989). **In the context of this paper, an indicator is a statistic or measure which facilitates interpretation and judgement about the condition of an element of the world or society in relation to a standard or goal** (modified from U.S. EPA, 1972).

Environmental indicators are selected key measures which reveal or summarize some aspect of the state of the environment, natural resource assets and related human activities. They focus on measures of environmental change and convey how the environment is responding to both stresses on the environment and management responses (Kerr, 1990).

2.2 Characteristics of Environmental Indicators. Criteria for selecting environmental indicators have been reviewed by several analysts (Gelinas and Slaats, 1989; Ward, 1990; among others). These are summarised below and have implications for the design of data collection and monitoring programs.

A. Policy Relevance. From a decision-making viewpoint, policy relevance is an essential feature of an environmental indicator. The utility of an indicator is a function of the extent to which it informs us of our progress in moving toward (or away from) established goals, targets or objectives (discussed in detail in the section on Reference Thresholds).

Cause/effect is a second factor related to policy relevance. Indicators which summarise information about environmental conditions or stresses where a cause-effect relationship has been established are less "ambiguous" than those where such a relationship is weak or poorly understood.

B. Detection of Temporal and/or Spatial Trends. As noted above, decision-makers, in deciding where to focus and allocate resources, require information on whether conditions are stable, improving or deteriorating and on how conditions in different areas compare with one another. A common problem is with deciding on where resources should be allocated and mitigating strategies applied.

By presenting information on environmental conditions and pressures using data which have been collected consistently over time and space, indicators can provide needed guidance to decision-makers.

C. Geographic Scope. There is a spatial continuum in which environmental indicators can be applied which ranges from the site-specific to the global level. However, the extent of geographic coverage will often influence the utility of an indicator, which generally increases with size of spatial coverage. For issues of national significance an indicator should provide a national picture of the phenomenon being reported.

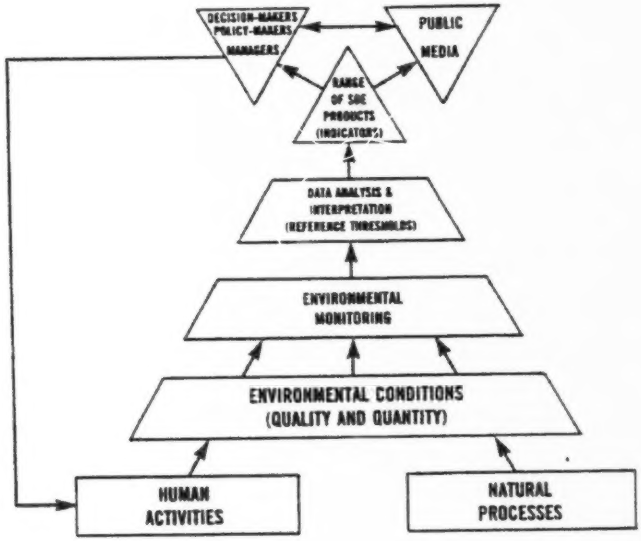
D. Scientifically Rigourous. Environmental indicators should be technically sound and their attributed significance should be scientifically defensible. There should be general consensus among credible experts regarding the validity of the indicator.

E. Understandable. Indicators should be easily understood by Canadians and by decision-makers. What the indicator represents and the significance of the values reported should be understandable. In this regard, the use of reference thresholds is especially helpful in communicating indicators and interpreting their significance.

3. ESSENTIAL BUILDING BLOCKS: ENVIRONMENTAL MONITORING AND REFERENCE THRESHOLDS.

Environmental indicators cannot be developed and applied in the absence of key building blocks; specifically, the availability of rigourous and consistent data, and thresholds against which to interpret reported values. The strategic importance of these elements is depicted in Figure 1, and is reviewed below.

FIGURE 1: The Relationship Between Monitoring, Reporting and Decision-making (modified from Kerr, 1990).


DECISION-MAKERS
POLICY-MAKERS
MANAGERS
PUBLIC
MEDIA
RANGE
OF SOE
PRODUCTS
(INDICATORS)
DATA ANALYSIS &
INTERPRETATION
(REFERENCE THRESHOLDS)
ENVIRONMENTAL
MONITORING
ENVIRONMENTAL CONDITIONS
(QUALITY AND QUANTITY)
HUMAN
ACTIVITIES
NATURAL
PROCESSES

3.1 Environmental Monitoring: Role and Design Considerations. Environmental monitoring can be defined as the repetitive observing of one or more environmental parameters according to pre-arranged schedules in space and time, using comparable methodologies for environmental sensing and data collection (O'Neil, 1990). The role of monitoring, simply stated, is the collection of data and information. As such, it is a key element in any environmental management or decision-making process. The data generated through monitoring can provide a sound scientific basis for management decisions. Monitoring can provide a record of changes occurring in key environmental indicators and help explain why such changes are occurring.
(the relationship between marine monitoring and decision-making is reviewed in detail by the National Research Council, 1990).

However, to maximize the utility and value-added of environmental monitoring, and to allow for the development of environmental indicators, several factors (among others) should be kept in mind (reviewed in detail by Philips and Segar, 1986; Wolfe, 1987; National Research Council, 1990). **First, the management objectives and information needs that monitoring is to address should be clearly identified.** In such a management-oriented approach to monitoring, social, economic and environmental values should be represented as directly as possible in the selection of parameters to be monitored (Wolfe, 1987). As a basis for making and priorizing decisions, monitoring information should inform us about whether important objectives are being met and uses sustained.

Second, the data generated should be comparable over time and space. Without such consistency temporal trends in environmental conditions cannot be identified and spatial comparisons cannot be made. This requires that the sampling protocols and analytical procedures inherent to the monitoring program be consistent.

A third and key consideration relates to the scope of monitoring. Is monitoring to be carried out on an integrated ecosystem-wide basis or is it to be more restricted in scope by focussing on selected issues and concerns (such as contaminant presence) ? This is a key question which must be addressed. The integrated approach involves ecosystem-wide monitoring where all elements of an ecosystem are surveyed for change in condition.

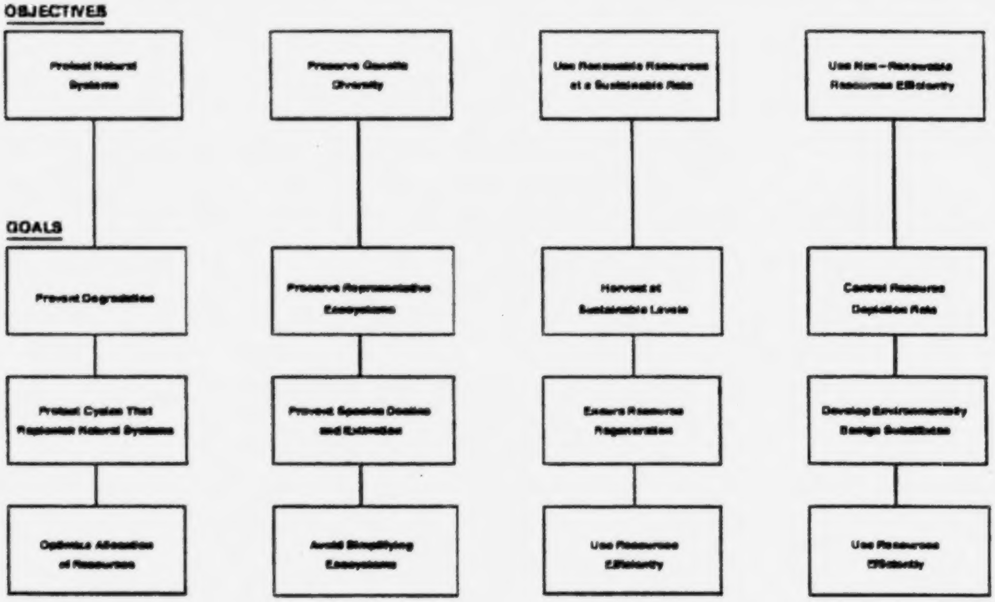
From a coastal pollution assessment perspective, the monitoring of contaminant fate and effect merits special attention. The presence and concentration of contaminants can be measured in three media: water, sediments and in living organisms. The extent of contamination, and the potential effects, cannot be gauged without recognizing that these three components act as a single system. Rather than choosing just one medium to monitor, information from all three should be incorporated to obtain an integrated view of contaminant presence and effect in the environment (Worf, 1980).

Monitoring for contaminant presence also has implications for monitoring program design. For decision-making and environmental reporting purposes, it is important that the data generated through monitoring reflect conditions at different contamination gradients. For investigations of temporal and spatial variability in contamination, a number of sites ranging from highly contaminated to background would be chosen, probably in inshore locations. In contrast, detection of long-term trends in environmental quality would require the monitoring of sites further offshore, removed from the influence of important contaminant sources (Philips and Segar, 1986). The "gradient to background" approach to monitoring was endorsed by a recent Environment Canada - Memorial University workshop on environmental effects monitoring (Environment Canada and Memorial University, 1988). Also important is that the monitoring sites be chosen so that they reflect the broad picture regarding the state of the environment. This is a problem given the statistical limitations of sampling design. Insufficient knowledge regarding the extent of contamination and impact introduces an element of chance into monitoring design which must survey and infer broader conditions from information collected at fixed locations. Shalski (1990) reviews this problem and suggests a method of rotational surveying which increases the statistical confidence associated with monitoring results.

3.2. Objectives and Reference Thresholds: Essential Interpretive Tools. Environmental indicators show whether or not objectives are being met, or progress made vis-a-vis identified goals. This approach implies that objectives be clearly identified prior to the identification of indicators. Objectives, in the sense meant here, are broad goals that policy-makers are striving to meet and that are generally deemed desirable to attain. One model of such objectives is presented in Figure 2:

Reference thresholds associate closely with objectives and aid with interpretation of data. Interpretation of data is an essential feature, and one of the truly value-added aspects of environmental reporting (Kerr, 1990). Raw data are meaningless to decision-makers and the public until summarised and interpreted, therefore methods of aggregating and presenting data over time and space, and of interpretation, are needed. Reference thresholds offer one way of interpreting data in a meaningfull way and, like objectives, are essential to indicator development. Environmental quality is a concept largely based on human values. Environmental objectives articulate these values while reference thresholds quantify them by identifying ranges of desireable/undesireable conditions or activities (eg. normal, problem, critical and irreversible) (Gelinas and Slaats, 1989). Environmental indicators juxtapose monitoring data against threshold levels of environmental quality and thus facilitate assessment of the state of particular conditions (ie. whether conditions are acceptable or unacceptable), and of whether they are improving or deteriorating (ie. moving toward or away from threshold levels of environmental quality).

FIGURE 2: Schematic Presentation of Sustainable Development Objectives (modified from Sheehy, 1989)


OBJECTIVES
Protect Natural Systems
Preserve Genetic Diversity
Use Renewable Resources at a Sustainable Rate
Use Non-Renewable Resources Efficiently
GOALS
Prevent Degradation
Preserve Representative Ecosystems
Harvest at Sustainable Levels
Control Resource Depletion Rate
Protect Cycles That Replenish Natural Systems
Prevent Species Decline and Extinction
Ensure Resource Regeneration
Develop Environmentally Benign Substitutes
Optimize Allocation of Resources
Avoid Simplifying Ecosystems
Use Resources Efficiently
Use Resources Efficiently

As denoted here, the term "reference threshold" is loosley used to denote any threshold against which monitoring data can be compared. The use of reference thresholds is portrayed in Table 1; examples of threshold "types" are depicted in Table 2.

TABLE 1: Example of the Relationship Between Objectives, Reference Thresholds and Indicators

Objective:	Prevent Environmental Degradation
Reference Threshold:	Standard for effluent toxicity
Indicator:	Measure of performance in meeting standard (indicator identifies whether or not effluent meets established toxicity threshold)

TABLE 2: Examples of Reference Thresholds.

Type of Threshold	Application
Environmental quality guideline, objective or standard	Contaminant levels in environmental media or effluents (eg. water quality guidelines, Apparent Effects Thresholds)
Activity target or goal	Target established as a policy goal (eg. DFO "no net loss" of habitat, Montreal Protocol Targets for phaseout of CFCs)
Comparative threshold	Data compared against background conditions or conditions at other reference sites.
Ecological Threshold	Contaminant levels which induce adverse biological effects (eg. eggshell thinning); minimum population size required for viability.

4. USING ENVIRONMENTAL INDICATORS: ILLUSTRATIVE EXAMPLES.

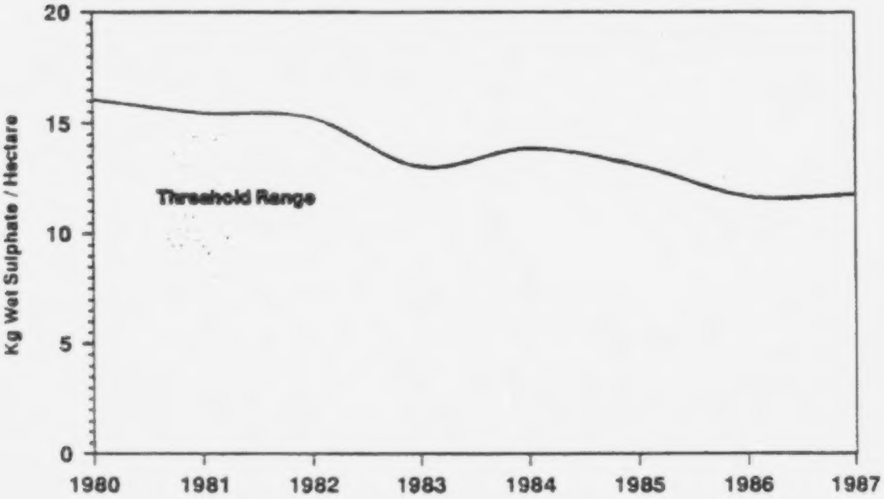
Environmental indicators can be grouped into two categories: single-measure indicators and composite indicators (sometimes referred to as indices). Most of what are commonly referred to as environmental indicators fall into the former category while many of the commonly reported economic indicators fall into the latter.

To illustrate the concepts outlined in previous sections of this paper two examples of indicators are presented: 1) acid deposition as an indicator of acidification stress on the environment; and 2) the Ontario Air Quality Index (AQI) as an indicator of environmental quality. These have been selected as representative of the two indicator types mentionned above (single measure, composite) and because they make effective use of monitoring data and reference thresholds to influence decision-making and public awareness. As such, each indicator is examined from the following viewpoints:

-- relation to a societal goal;
-- monitoring and data support, including spatial and temporal coverage;
-- use of reference thresholds;
-- policy relevance and utility to decision-making.

4.1 Example Indicator -- Acid deposition. Acidification of the environment is a regional problem which has affected much of the central and eastern Canadian environment. Environmental indicators have been used to assess and portray the extent of the problem and to track progress achieved in resolving it.

FIGURE 3: Example Indicator -- Trends in Wet Sulphate Deposition: Eastern Canada (M. Still, Pers. comm.)



Societal goals. Resolution of the acid rain problem speaks to two broad goals (as outlined in Figure 2):

- Protection of natural systems and prevention of degradation;
- Preservation of biological diversity and prevention of species extinction and decline.

Monitoring and Data Support. This indicator is constructed from and supported by an extensive data collection and management system -- the Canadian Air and Precipitation Monitoring Network (CAPMON). An array of parameters associated with the acid rain issue are monitored daily at 24 stations in rural areas from all provinces and territories save Yukon and P.E.I.. Monitoring is site specific at regionally representative sites. The indicator can be presented for specific sites or summed and portrayed over larger areas. Sampling and analytical procedures are intercalibrated and the system allows for detection of spatial and temporal deposition trends.

Use of Reference Thresholds. Reference thresholds have been developed which allow the data collected from CAPMON to be communicated and used through an indicator. Through scientific research a threshold level ranging between 8 and 15 kg per hectare of wet sulphate deposition has been identified (exact amount is a function of local area characteristics). Deposition above this level will likely result in damage to aquatic and terrestrial ecosystems (eg. reduced biodiversity and productivity).

Policy Relevance and Use for Decision-making. Policy-makers have made extensive use of this indicator. Related indicators such as aquatic pH and loss of biodiversity signalled in the 1970s that acidification was a problem requiring urgent attention. Establishment of a threshold level of sulphate deposition and identification of the gap between current and desireable conditions led to the formulation of an emission reduction program designed to decrease sulphate deposition loadings into the environment. By tracking the wet sulphate deposition indicator, progress made in attaining stated goals can be monitored and the success of recent acid rain control policies evaluated.

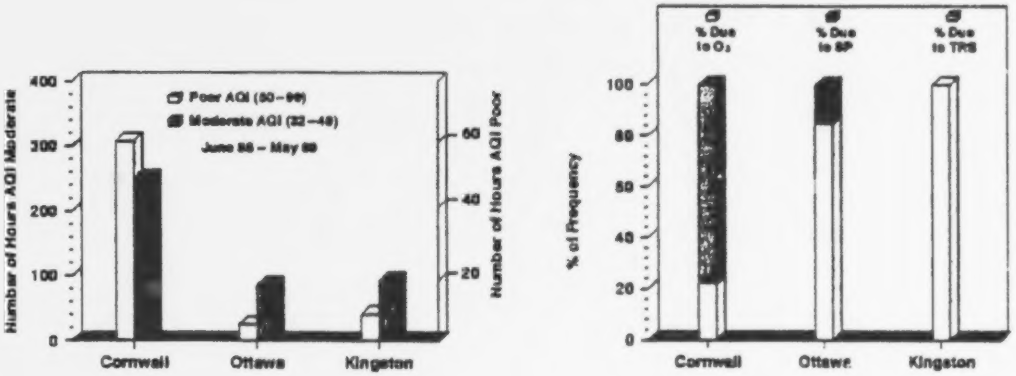
4.2 Example Indicator -- Ontario Air Quality Index. An index reduces complex and voluminous monitoring data for an array of parameters into a single number which can be used to give an indication of overall environmental quality (Federal - Provincial Committee on Air Pollution, 1980). There have been few quantitative indices developed for environmental quality; only those related to air quality have achieved widespread use in Canada (some countries, like the United Kingdom and New Zealand, have developed water quality indices). The Ontario Air Quality Index (AQI) is a good example of such an index. The index is used as a tool to better inform the public on the quality of Ontario's air and reports this quality in the following unit-free numerical classifications.

0 - 15	very good
16 - 31	good
32 - 49	moderate
50 - 100	poor
> 100	very poor

Societal Goals. The goals which the index relate to are the protection of human health from air pollution and the protection of natural systems by minimizing air pollution effects on the environment.

Monitoring and Data Support. The data used to derive the AQI are obtained from a monitoring network consisting of 33 monitoring sites in 26 cities across the province. The system provides hourly information for six air pollutants (sulphur dioxide, suspended particles, nitrogen dioxide, ozone, carbon monoxide and total reduced sulphur compounds. Air quality samples are collected and analysed in a consistent manner allowing for spatial and temporal comparisons.

FIGURE 4 : Ontario Air Quality Index (Yap et al., 1989)



Use of Reference Thresholds. Reference thresholds are essential to the development of the index and have been established for each of the six pollutants in the index (Federal and Ontario Air Quality Standards). The hourly concentration of each of the pollutants is calibrated to a common sub-index scale ranging from zero upwards (the same scale as the "quality" categories presented above). The sub-index is calculated for each pollutant based on its effect and the highest sub-index at any time becomes the value of the overall AQI.

Policy Relevance and Use in Decision-Making. The AQI is one of the most widely reported environmental quality indicators. The AQI levels for each city are reported several times daily to the news media and there is much interest from the media and the public when the air quality is in the Moderate and Poor ranges.

From a decision-making viewpoint, the index can, and does, report the number of times each pollutant is responsible for values of the index falling in the moderate to poor ranges of air quality. Armed with this information, policy-makers can direct resources toward lowering levels of the most critical air pollutants. Decisions can be taken to temporarily close air pollution sources such as power plants if air quality conditions deteriorate to critical levels.

5. CRITIQUE OF EXISTING DATA AND IMPLICATIONS FOR MARINE ENVIRONMENTAL QUALITY MONITORING.

A 1985 program review of major surveys in Canada found that many were costly and inefficient. It criticised the overall system of environment and natural resources monitoring as lacking a clear focus and integration of their activities. The review concluded that "in those survey departments having a scientific research sector, the national policies and objectives for the science are not always clearly articulated to management" (Canada Task Force on Program Review, 1985).

Generally, many of our environmental monitoring programs were developed and implemented years ago when the very nature of environmental issues and management needs was substantially different. In the past, concerns focussed on relatively simple environmental problems and monitoring was largely oriented to single issues and single-media surveys. The complexity of today's issues requires a more comprehensive understanding of pressures on the environment and environmental change (Kerr, 1990).

This paper reviews how monitoring, reference thresholds and indicators can be used to effectively develop and apply environmental information for decision-making purposes. But what form of marine environmental monitoring is required to meet today's marine management needs ? **For environmental reporting, with its focus on cross-sectoral analysis, and for marine management, an ecosystem approach to monitoring is recommended.**

Marine ecosystems are dynamic entities which provide an array of living and non-living resources, support a variety of (often conflicting) uses and have tremendous ecological and intrinsic value. Comprehensive ecosystem monitoring, in the marine context, would involve monitoring of human activities with the potential to adversely affect marine ecosystems (eg. contaminant inputs, fishing), components of the marine environment at varying levels of organisation (eg. coastal habitats, populations of marine species) and the concentration and effects of contaminants and pollutants (Table 3).

The desired scope of marine monitoring is thus an important question to consider. Other considerations include the adequacy of existing Canadian marine monitoring relative to management needs, and whether the data generated by such programs are nationally consistent and comparable. A better knowledge of what is being monitored in the marine environment, where, and by whom is needed. These questions, among others, should be addressed as part of the larger process of developing a national marine environmental monitoring program in Canada.

TABLE 3: Elements of Integrated Marine Monitoring

A. Pressures on the Marine Environment

-- contaminant inputs
-- coastal land-use
-- harvesting activity

B. Environmental Condition/State

-- concentrations of contaminants (water, sediments, biota)
-- Biotic state (biomass and population change, species health, bio-diversity)
-- ecosystem change (eg. productivity)

Recent Canadian reviews of marine environmental quality (Kay, 1989; Environment Canada, 1989) concluded that existing data allowed for a snapshot assessment of the state of the marine environment but could not support any firm conclusions regarding whether or not conditions were stable, improving or deteriorating. Requests by international organisations like the Organisation for Economic Co-operation and Development (OECD) for national-level data on marine environmental quality cannot be adequately met. The problems with existing marine data can be summed as follows:

-- data often result from limited monitoring at specific sites. Such monitoring may well meet the needs it was intended for, however it often precludes temporal analysis of trends and spatial comparison among regions;

-- sampling and analytical protocols often differ, even within departments or agencies. Thus even if two groups are monitoring similar parameters results may not be comparable;

-- there are gaps in the coverage of monitoring. A full analysis of existing monitoring relative to identified management needs would determine more precisely critical data gaps; however, experiences to date point to gaps in, amomng other things, pollutant inputs (an input "budget" cannot be developed for the

Canadian marine environment from existing data), contaminant distribution and effect and compliance with environmental standards and regulations.

6. SUMMARY OF KEY CONSIDERATIONS

Environmental reporting in Canada is still in an early stage of development. Many of the monitoring programs and data sets upon which current reporting efforts rest were designed to respond to site-specific and sector-specific requirements and issues. Much work remains to be done to identify the indicators which should be tracked, to put in place the monitoring and data support systems which will allow this to be done and to develop the reference thresholds which will facilitate interpretation of data. This is true of the marine environment as well as of other environmental sectors.

As work proceeds towards the elaboration of a marine monitoring programme, the following steps should be addressed:

1. National and regional marine management objectives and issues for which information is required should be clearly identified;

2. Reference thresholds which identify desired levels of environmental quality should be developed. To the extent possible, these should be quantitative and linked with management objectives;

3. The information required to identify whether thresholds are being achieved and objectives met should be determined. Existing monitoring efforts which meet objectives should be identified and evaluated, and gaps pin-pointed. Monitoring programs required to address information gaps should be designed and implemented. Consideration should be given to ensuring national and regional spatial and temporal consistency.

4. Management information is produced only when it is delivered to managers and decision-makers in a usable, accessible form (National Research Council, 1990). Using indicators, information on trends in marine environmental quality and on areas and issues of concern should be reported in an understandable and systematic way to decision-makers and the public.

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MONITORING THE COASTAL AND ESTUARINE HEALTH OF THE UNITED STATES USING BIVALVE MOLLUSKS AS SENTINEL ORGANISMS. G. G. Lauenstein, National Status and Trends Program, Ocean Assessments Division, National Ocean Service, NOAA, 6001 Executive Boulevard, Rockville, MD 20852, USA (301-443-8655).

The U.S. National Oceanic and Atmospheric Administration uses bivalve mollusks to monitor national estuarine and coastal health. The project was initiated in 1986 and has collected mollusks and associated sediments annually since that time. Three mollusk species (*Mytilus edulis*, *Mytilus californianus*, and *Crassostrea virginica*) are used to quantify more than 70 contaminants along the conterminous United States and Alaska coasts while the use of an additional species (*Ostrea sandvicensis*) is necessary for the Hawaii islands.

With data available from 1986-1989 it can be concluded that concentrations of heavy metals and organic contaminants are highest in urban areas. Also, for certain estuaries both heavy metals and organic contaminant concentrations and associations are specific to the given estuary. Analysis of the data for possible temporal trends indicate that at least some contaminants may be decreasing at certain sites while increasing at others.

MONITORING ALGAL BLOOMS CAUSING SHELLFISH POISONING AND FISH KILLS.

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Blooms of toxic species of algae can cause the death of marine fauna or harm humans when contaminated shellfish or fish are consumed. Several groups of algae and a variety of toxins, both water- and fat soluble, are involved and it is unlikely that any coastal area throughout the world is free from them. Aquaculture activities have contributed to an increase in the incidence of reported outbreaks, chiefly through the increased amount of product exposed to such blooms and widespread marketing, but there are concerns that the farming activities themselves could lead to the increase in blooms through eutrophication.

In British Columbia the chief problems are paralytic shellfish poisoning in both wild and cultivated bivalves, produced by several species of dinoflagellates of the genus *Protogonyaulax* (= *Alexandrium*), and kills of farmed salmonids by chloromonad flagellates (*Heterosigma* plus a new, undescribed form) and diatoms with barbed setae.

Blooms of such organisms are natural events and cannot be prevented although their impact can be reduced through monitoring of the plankton and other key environmental parameters. The paper outlines some basic features of such programs, with particular reference to British Columbia.

RIVERINE INPUTS IN MARINE ENVIRONMENTAL QUALITY MONITORING.

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The contribution of rivers is an important part of marine environmental quality monitoring because rivers may represent an important source of contamination to the marine environment. Riverine input is especially important in coastal areas with restricted circulation and large river flows such as some bays and estuaries.

The riverine flux of a contaminant may be calculated as the product of the riverine concentration of the contaminant and the river water discharge. To understand the true riverine flux of a contaminant, one must know the actual values of both the contaminant concentration and the river flow and understand how each is affected by anthropogenic and natural processes.

In the past, there have been some large programs to measure the quality of many rivers, and some of the data from these programs is being used to project trends in stream quality and fluxes of contaminants to the coastal zone. But how reliable are these data? Marine chemists have recognized for years that contamination is a problem in the sampling, handling and analysis of environmental samples, especially the ultra-trace concentrations found in open ocean waters. Since there has been a lack of standard reference materials, marine chemists have had to rely upon intercalibration exercises to assess the quality of their methods. A series of intercalibrations of dissolved metals in seawater have resulted in vastly improved results for the analysis of trace metals in seawater in recent years.

The United States Geological Survey has been collected a large database of dissolved trace metal concentrations in rivers as part of the National Stream Quality Assurance Network (NASQAN). During a study of global river geochemistry, we sampled most of the large rivers along the east coast of the US and Canada, along with some smaller rivers. Some of these sampling sites were near sampling stations that are included in the NASQAN network. Thus, we were able to compare our data with those in the Nasqan database for the same sampling period.

We used clean sampling and handling techniques during periods of high and low flow and averaged the data to represent a mean concentration. Our data for Cu, Cd, Pb and

Zn are consistently lower than those reported in the NASQAN database, Cd, Pb and Zn being 1 to 2 orders of magnitude lower (Windom, et al., 1990). Our results are similar to those obtained by other investigators (Schiller and Boyle, 1985; 1987), which leads us to believe that the NASQAN data are not suitable in determining riverine fluxes or trends in trace metal concentrations.

It is also necessary to assure that data being reported by different laboratories in an environmental quality monitoring program are comparable. The best method for determining this is by intercalibration exercises. Even with the higher concentrations usually found in rivers, it is necessary to perform these types of exercises.

Results from a recent intercalibration of marine sediments that was performed among laboratories in Asia and the United States, show that even for elements that are relatively easy to sample and analyze, such as iron and manganese, there can be large systematic variations among labs.

If we are assured that the data represent true concentrations, are these concentrations the result of natural or anthropogenic processes? Our results for East Coast rivers suggest that although dissolved cadmium and zinc appear to be enriched in these rivers, these elements vary systematically with pH. The same holds for dissolved lead, although the lead concentrations in some rivers may reflect a depletion of lead in the soils of the drainage basin due to extensive weathering. The conclusion is that the dissolved concentrations of these trace metals may be influenced more by the chemistry of the rivers than by anthropogenic sources.

Given the particle reactive nature of many trace metals (and synthetic organic compounds) and the effects of river chemistry upon dissolved concentrations, the concentrations of trace metals on suspended particles may be a better method of assessing environmental quality than dissolved trace metal concentrations. If we calculate an enrichment factor for trace metals on suspended particles in East Coast rivers versus average crustal material, we see that enrichments are best seen on particles from rivers having small suspended loads. In rivers with large concentrations of suspended material, the enrichment factor is low and relatively constant, reflecting dilution of contaminants by natural material.

If we trust the data to represent the true trace metal concentrations of a sample, can we use the concentrations with discharge data to calculate the real flux? The concentrations of riverborne materials often vary with the discharge. This can often be estimated by a rating curve, an equation of the form $C = aQ^b$ where C is the concentration, Q is the discharge, and a and b are constants. These rating curves may vary dramatically among rivers (GESAMP, 1987, and references therein), so that contaminant concentrations may be greatly affected by changes in river flow.

Storm related events have a large impact upon the transport of material by rivers and may account for the majority of the annual riverine flux of a substance. The concentrations of some constituents vary with discharge, some reflect dilution by the larger water flow, while some increase through the storm period, reflecting a flushing effect of material stored in the drainage basin. Depending upon the intervals between storm events, antecedent effects from previous storm events may reduce the amount of transport in successive storm events.

It is necessary to understand the magnitude of riverine inputs in any study of coastal marine environmental quality. One must be assured that both past and present riverine data is both reliable and comparable. Natural processes, including temporal changes in riverine fluxes, must be understood to discern variations in environmental quality that are due to anthropogenic influences.

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STATISTICAL CONSTRAINTS OF STATUS AND TRENDS ANALYSIS OF ENVIRONMENTAL QUALITY DATA: TRENDS IN CONTAMINANT LEVELS IN FISH.

EXTENDED SUMMARY

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INTRODUCTION

Investigation of time trends in contaminant levels in fish stocks has been of primary interest to the International Council for the Exploration of the Sea (ICES) since 1974. Conventional analysis of variance (ANOVA) and analysis of covariance (ANCOVA) have been widely used for analyzing data on contaminant levels (ANOVA) or contaminant levels with length as the covariate (both transformed to logarithms) (ANCOVA), measured on individuals from several years of collection. ANCOVA is an inadequate procedure when regression slopes (coefficients of regression of contaminant concentration on length) for separate years and residual variances about these separate regressions are not equal for all years. By employing a weighted procedure (Misra et al. 1990) this methodology is extended to analyze such data sets where homogeneity of regression coefficients and residual variances is not present.

It must be further recognized that: 1. The scope of a univariate analysis is restricted because it analyses data on only one contaminant at a time. Thus, a series of univariate ANCOVAs will ignore the mutually correlated structure of the dependent (Y_i) variables. Multivariate analysis of covariance (MANCOVA) will not ignore correlations among Y_i variables as data on all contaminants are analyzed jointly. Information about relationships, interdependence, and relative importance of variables is retrievable from the MANCOVA. The ANCOVA model is fully absorbed in the MANCOVA model. 2. Frequently, analysis of real data has shown the need for more than one covariate to be employed, e.g. for approximately one-half of 62 contaminant trend data sets collected under the Cooperative Monitoring Programme of the ICES, statistically improved analyses were achieved when more than one biological covariate (length) was employed. Year-means must be adjusted for variations in all available biological covariables. Biological covariables are generally mutually correlated. In particular, biological characters such as length, weight, age, are highly correlated as they are all related to fish size. Although several techniques are available for handling this problem of multicollinearity, no technique works well in all circumstances or is without constraint. The approach we employ to overcome the problem of multicollinearity (Misra et al. 1989) uses principal components (PCs) of biological covariables within the MANCOVA of Canadian Atlantic cod data — the PC-MLR method.

WEIGHTED ANALYSIS OF COVARIANCE

The weighted procedure presented here assumes that only one covariate is in use. The procedure can be extended to the case where two or more covariates are employed. The general problem in many circumstances is to examine how a quantity is varying on a medium- to long-term scale when its concentration (Y) is correlated with an associated variable (X), the influence of which is not fully controllable by sampling. Its concentration is, in any case, likely to vary between years as other, associated parameters vary. Comparison of multiple (K) means is potentially difficult in any case. Our study was undertaken to find an acceptable way of analyzing data sets that are not amenable to the conventional ANCOVA. Several procedures for multiple comparison of means exist (e.g. see Miller 1981). There is not complete agreement on how best to conduct multiple comparisons (Harris 1975) or even on the general principles of multiple comparisons (Miller 1981).

Also, poor data structure, a characteristic of many monitoring programs (ICES 1989), would induce irregularities due to improper definition of the effects of the covariate on contaminant concentration levels. Consistency of a regression coefficient depends not only on the sample size but also on the widths of ranges of the covariate for individual years. Data with wide ranges of the covariate have the following additional advantage (Li 1965): Points with covariate values near their mean ($\bar{X}$) will not necessarily prevent the regression line from rotating. On the other hand, points with large deviations from the mean and thus located towards either end of a wide range will tend to stabilize the line, as these points are much more effective in preventing the line from rotating. Sometimes, for practical reasons, close adherence to the sampling guidelines may be impossible but its importance should never be underestimated. Homogeneity of regression coefficients among years is still not assured as further causes of variation (other than the sampling procedure) in the values of the estimate, b_i, of regression coefficients also exist. For example, the ANCOVA model of the ICES guidelines employs only one covariate (X). It was also felt (ICES 1986b) that ignoring other covariables is not prudent, especially when some studies have shown the necessity of taking their effect into account. The ICES model with several covariates and the model with only one covariate, length, were fitted to 53 CMP data sets and their fits compared based upon their residual standard deviation values (ICES 1987b; Nicholson and Wilson 1987). The model with a single covariate gave increased residual standard deviations in about half the cases. In two cases, the increase exceeded 20% (ICES 1987b). In a similar analysis of contaminant data from Atlantic cod (*Gadus morhua*), Misra et al. (1989) reported increases in residual standard deviations of up to 107% for contaminant concentration data and up to 328% for contaminant burden (contaminant concentration times organ weight) data.

Biological variables available as covariates, e.g. length, weight, and age are frequently mutually correlated as they are related to fish size/growth (ICES 1989, 1987a). Omission of other covariates, which are correlated with the covariate X, employed in the regression, will bias the value of b_i (Draper and Smith 1981, Bliss 1970). It is further noted from Draper and Smith (1981, Chapter 6) that screening of the correct suite of covariates is not an easy task. Sometimes more than one "best" suite can be identified. Several techniques are available for this purpose and yet no technique works well in all circumstances, or is always better than all others. The Working Group on Statistical Aspects of Trend Monitoring (ICES 1989) noted that when covariates are correlated, the probability of selecting the covariates by a stepwise regression approach (which is considered to be one of the best of the variable selection procedures; Draper and Smith 1981) is low. Second and higher order associations (interactions) of omitted covariates alter value(s) of the coefficient(s) of regression on the covariate(s) retained in the regression model (Bliss 1970). The value observed when a biological character is measured on an individual may be referred to as the "phenotypic value" of that individual (Falconer 1972).

The above observations concerning statistical correlations between biological covariates relate to their phenotypic correlations (Falconer 1972). Further probing into the possible causes of correlation between variables requires extension of the phenotypic correlations to genetic and environmental correlations and to the examination of several possible causes of variations in these (Falconer 1972). A good sampling strategy, which includes a prudent choice of the study area, e.g. choosing a stable stock as opposed to an erratic or widely spread population, will help to stabilize the correlation between variables from one year to the another; but variances and covariances of biological characteristics are also properties of populations and of the environmental circumstances to which individuals are subjected (Falconer 1972). In actual studies the occurrence of heterogeneity of statistical parameters among years is, therefore, to be expected. At this time the true mathematical model incorporating varying b_j values, if there is one, is unknown. In the real world, therefore, we can expect to encounter data sets where β_j values are not the same for all years. We further note that often only a single covariate and dependent variable is available. Thus, some model for the efficient analysis of such data sets is needed.

The weighted ANCOVA methodology was developed from Neter et al. (1985), Snedecor and Cochran (1980), Press (1972), Armitage (1971), Bliss (1970), and Li (1965). In an ANCOVA for contaminant concentrations, year-means of Y are adjusted to a common value, A, of the covariate, X. These adjusted means are then analyzed (a) to test the general null hypothesis (H_{0A}) that these means are all equal, and (b) to test the specific null hypothesis H_{0L} of no time trend. Any specific value, A, may be chosen for the covariate, X. The most frequently chosen value is the grand mean of the covariate (Li 1965). An ideal example is shown in Figure 1. The adjusted mean of Y for group (year) j is simply the ordinate of its regression line at X = A. In the general case where K regression lines are not parallel, e.g. Figure 2, (i.e., variation from group to group in b_j is not within the sampling error), the year effect can only be studied by comparing the regression lines for each year (Neter et al. 1985) and the test of H_{0A} that adjusted year-means are all equal would depend upon the value of A selected.

Two weighting procedures are reported (Misra et al. 1990), based on results of tests of equalities of slopes and variances, in particular, a general one for use when heterogeneity of σ_j^2 and β_j values exists. The test for a time trend is as follows:

Define linear comparison

$$\hat{Z} = \sum_{j=1}^{K} M_j \bar{Y}_{Aj} \qquad \text{where} \quad \sum_{j=1}^{K} M_j = 0$$

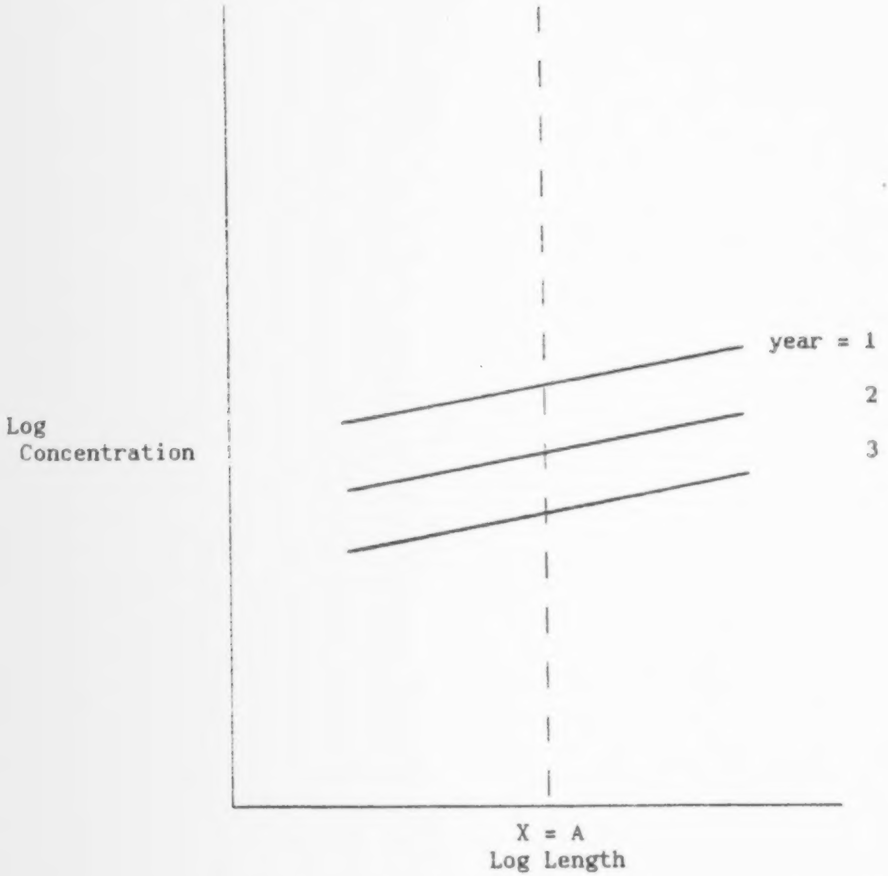

Figure 1. Regression lines for K groups (years) — Ideal Situation,

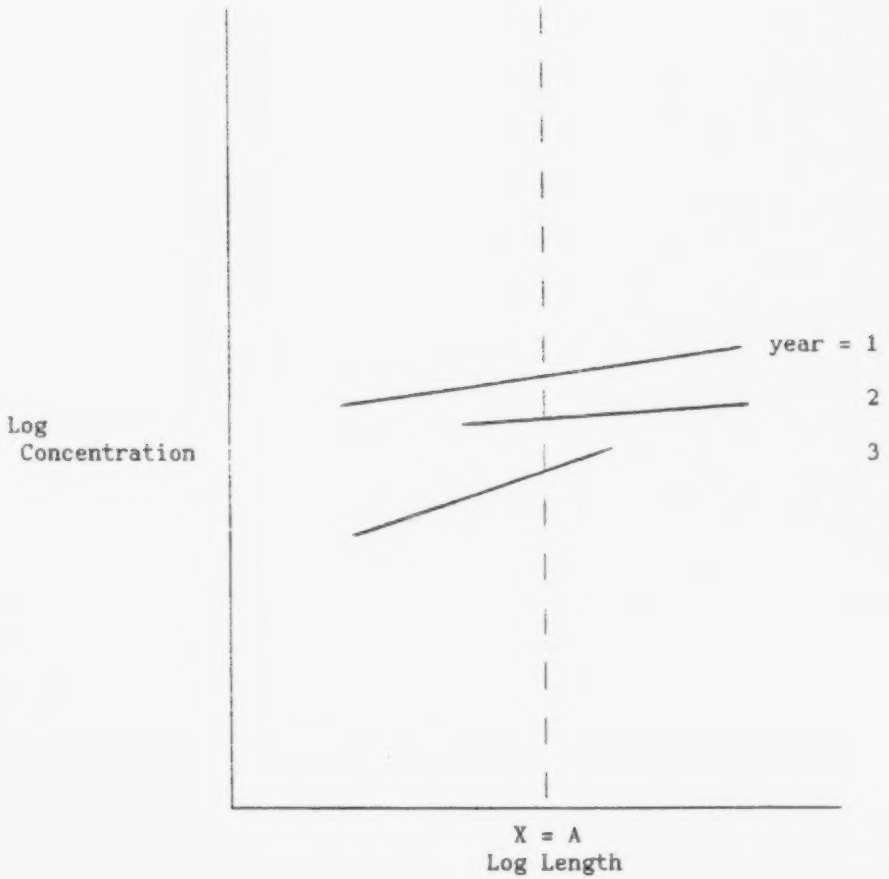

Figure 2. Regression lines for K groups (years) — Example of Real Situation,

for a time trend by providing appropriate values of M_j from a table of orthogonal polynomials where years are equally spaced, or by calculating their values by the method explained in Bliss (1970) when years are not equally spaced. Calculate SS due to this linear comparison as

$$SSL = (\sum_{j=1}^{K} W_{Aj} M_j \bar{Y}_{Aj} - \sum_{j=1}^{K} W_{Aj} M_j \cdot \sum_{j=1}^{K} W_{Aj} \bar{Y}_{Aj} / \sum_{j=1}^{K} W_{Aj})^2 / (\sum_{j=1}^{K} W_{Aj} M_j^2 - (\sum_{j=1}^{K} W_{Aj} M_j)^2 / \sum_{j=1}^{K} W_{Aj})$$

Null hypothesis H_{OL}: $Z = 0$ for time trend is then tested.

The procedure was applied to a set of time trend data (flounder and cod from the Belgian coast). As heterogeneity of σ_j^2 and β_j values existed, temporal variations of adjusted year-means of Y and their time trends were analyzed by the general weighted method. Figure 3 gives time trend lines for the six data sets. The following benefits were found:

1. All S.E. values of the common regression coefficients were smaller when weights (equation 9) were employed.

2. All common regression coefficients except the regression coefficient for Y_2 of cod were significant when weights were employed.

MULTIPLE ANALYSIS OF COVARIANCE USING PC-MLR APPROACH

Neter at al. (1985) and Draper and Smith (1981) discuss the problem of correlated regressor (independent) variables. Although all possible regressor variables are desirable in the MLR, the use of a large set of variables presents a multitude of difficulties, including the occurrence of highly significant, yet erroneous findings. The use of principal components (PCs) as working variables offers several advantages. There is no loss of information if the set of X_j variables is replaced by their PCs (Harris 1975). Frequently, with biological data the information underlying the p variables X_j is summarized satisfactorily in a small number (q) of their PCs. PC analysis provides a means of discovering the fact of linear dependence of X_j variables and specifying the nature of the dependence. In PC-MLR with q PCs, the combined effect of q partial regression coefficients is partitioned orthogonally. Thus PCs can be used as covariates either singularly or simultaneously in the MANCOVA.

The MANCOVA is explained in Srivastava and Carter (1983) and Morrison (1976) and employed to analyze data on Canadian cod in Misra and Uthe (1987). The PC-MLR approach is described in Misra et al. (1989) with a full account given in Johnson and Wichern (1988), Draper and Smith (1981), and Morrison (1976). PCs were developed from the covariance matrix S of X_j with p eigen value-eigen vector (λ_K - Z_K) pairs. The principal component transformation is an orthogonal transformation which rotates the coordinate axis of the original X_j variables to the axes of the PCs which have the following features: 1. these PCs are uncorrelated with each other; 2 variability of the total system is associated with PCs in decreasing order. Therefore, it is possible that the first one or two PC variables are sufficient to summarize the bulk of the variability and covariability of the original X_j variables. PC coefficients are scaled to make Z_K of unit length. Draper and Smith (1981) and Morrison

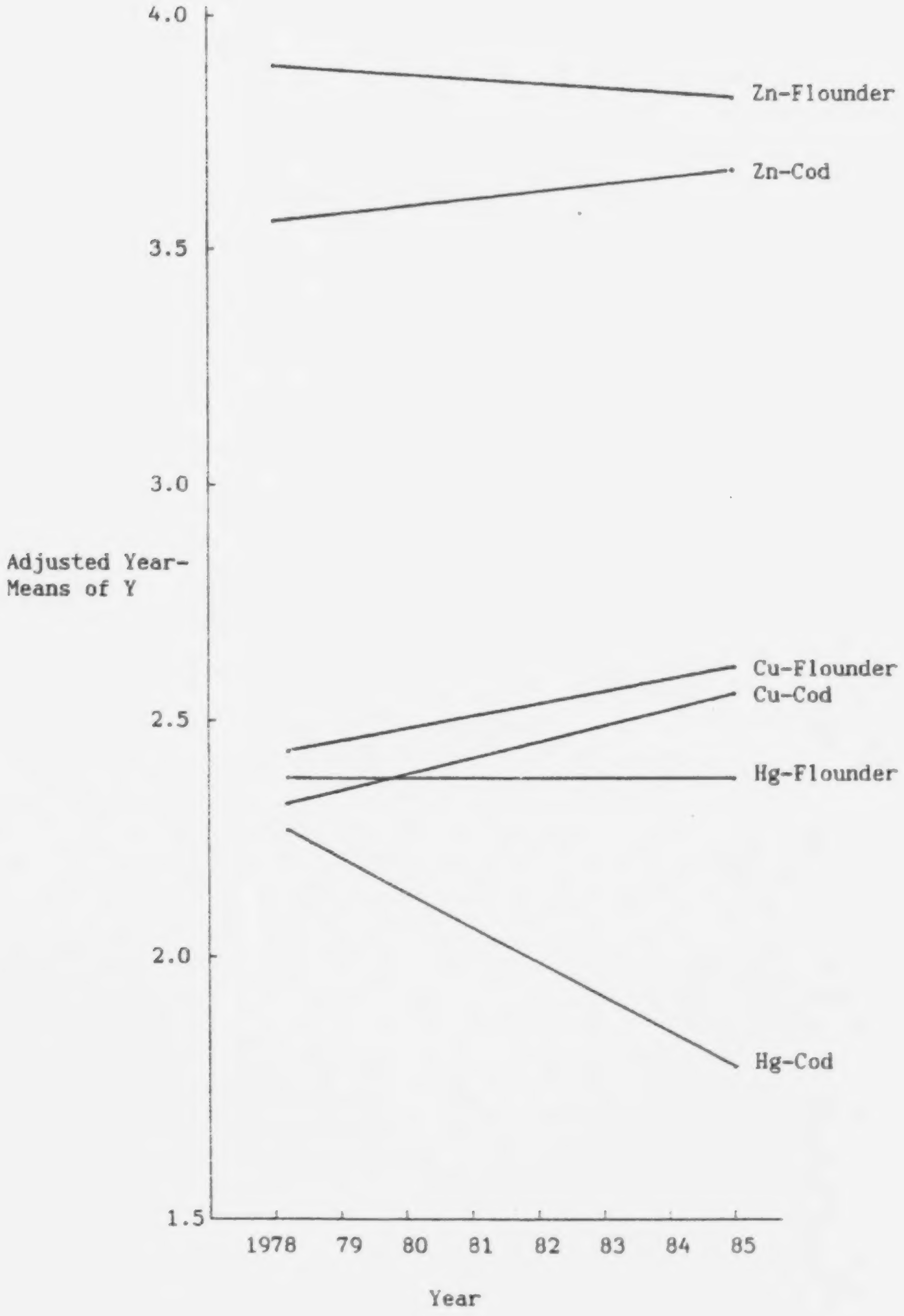

Figure 3. Time trends for Zn, Cu and Hg in Cod and Flounder of the Belgian coast, 1978-1985.

(1976) suggest that the first few Z_Ks which explain 75% or more of the total variance will adequately replace the p variables X_j.

The approach was applied to Canadian cod contaminant concentration and burden (concentration times the weight of the whole tissue) data (Misra et al. 1989). For concentrations data, two eigen values explained 81.4% of the total (standardized sample) variance, while 82.6% of the total standardized variance in the burden data was explained by the first eigen value. Linear time trends were significant although significant deviations from the linear trend were also present.

DISCUSSION

Trend analysis by either of the two above methods was shown to be superior to the more common methods of ANCOVA or MLR with multiple independent variables. The PC-MLR approach offers a method of overcoming problems associated with multicollinearity as well. Analysis of real data sets showed the feasibility of detecting significant time trends of relatively low magnitude within a population of rather diverse characteristics.

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MARINE MAMMALS AS INDICATORS OF ENVIRONMENTAL CONTAMINATION BY PCBs AND DIOXINS/FURANS.

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INTRODUCTION

It has been recognized since the late 1960's that marine mammals can accumulate high concentrations of lipophilic organochlorine pollutants in blubber and that monitoring of these populations would assist in identifying areas of contamination (Holden 1972). In Canada, detailed studies of PCB and organochlorine (OC) pesticides residues in marine mammal tissues began in the early 1970's with investigations of concentrations in Gulf of St. Lawrence harp seals (Addison et al. 1973; Jones et al. 1976) and Bay of Fundy harbour porpoise (Gaskin et al. 1971). Ringed seals and beluga in the western Canadian Arctic were also investigated (Addison and Smith 1974; Addison and Brodie 1973). Compared to ringed seals in the Baltic Sea (Helle et al. 1976) and harbour seals from the Dutch Wadden Sea (Reijnders, 1980), harp and ringed seals had low levels of PCBs and DDT-related compounds. Harbour porpoise from the Bay of Fundy, had among the highest DDT levels ever reported in cetaceans, probably resulting from the use of DDT for forest spraying during the 1960's in New Brunswick and Maine.

These early studies dealt mainly with DDT and PCBs and was limited by the technology available (packed column gas chromatography (GC)) to separate individual isomers of PCBs and OC pesticides. Over the past five years our laboratories have collaborated in the analysis of marine mammal samples from Canadian waters, using high resolution capillary GC. The list of OC contaminants found in marine mammal tissues now includes about 60 PCB congeners, as well as chlordane-and DDT-related compounds, toxaphene or polychlorinated camphenes (PCCs), mirex, and chlorobenzenes, hexachlorocyclohexanes, and polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

RESULTS

Results for PCBs, two major OC pesticides, and three major PCDD/PCDF congeners are summarized in Table 1. The summary includes most of Canada's resident cetacean (odontocetes) populations as well as Arctic ringed seals and polar bears.

Belugas from the St. Lawrence River estuary have the dubious distinction of having the highest PCB levels of all Canadian marine mammals analysed to date. DDT and PCCs were also prominent OCs in beluga fat, however, PCDDs and PCDFs are near detection limits (Table 1). Killer whales from Georgia Strait/Vancouver Island have the second highest PCB levels and are distinguished from beluga by higher levels of 2,3,7,8-TCDF. A false killer whale from Georgia Strait had 1920 μg/g total DDT (ΣDDT), mainly as the metabolite 4,4'-DDE, the highest level in any of the marine mammals analysed.

Harbour porpoise from Georgia Strait also had the high PCDD/PCDFs (Table 1). The highest level of 2,3,7,8-TCDD in the marine mammals surveyed was found in blubber of ringed seals from Barrow Strait in the central Arctic archipelago. White-beaked dolphins from the Gulf of St. Lawrence also had high DDT, PCC and PCB levels.

DISCUSSION

The results are discussed below in relation to their usefulness in assessing geographic and temporal trends in contaminants, and biological effects, and the capability of marine mammals to biotransform some contaminants.

Information on levels of contaminants in marine mammals tissues is perhaps most useful as an indicator of general geographic and temporal trends of contaminants in marine food chains. The results for polar bears in Table 1 are

Table 1. Organochlorine contaminants in marine mammal fat (fresh weight) from Canadian waters.

Species	N	Sex[1]	Location	Mean concentration ($\mu g\ g^{-1}$ ± SD)			Mean Concentration (ng/kg)			References[4]
				ΣDDT	PCC	ΣPCB[2]	TCDD	TCDF	HxCDD[3]	
Polar bear	18	P	Cornwallis Is.	0.30	ND[5]	5.94	20[6]	<2	<4	A,B
	10	P	North Baffin Is.	0.21	ND	4.22	4	<2	<4	
	10	P	W. Davis Strait	0.39	ND	3.24	3	<2	<4	
	20	P	South Baffin Is.	0.41	ND	4.25	5	<2	<4	
	9	P	W. Hudson Bay	1.19	ND	8.02	2	<2	<4	
Ringed seal	10	M	Cumberland Sound	0.33 ± 0.19	0.38 ± 0.16	0.51 ± 0.23	8	4	<8	A,C
	8	M	W. Davis Strait	0.41 ± 0.19	0.25 ± 0.16	0.54 ± 0.21	11	3	<8	
	16	M	Barrow Strait	0.71 ± 0.41	ND	0.57 ± 0.29	37	4	9	
Narwhal	16	M	Pond Inlet (Baffin Bay)	5.92 ± 1.71	12.2 ± 2.44	5.18 ± 1.34	ND	ND	ND	D
Beluga	6	M	Cumberland Sound	6.83 ± 1.89	13.1 ± 3.75	4.91 ± 0.25	<2	<2	5	A,C
	8	M	Jones Sound (Baffin Bay)	1.96 ± 0.32	4.25 ± 1.02	2.53 ± 0.57	ND	ND	ND	
	11	M	St. Lawrence estuary	96.1 ± 56.1	25.5 ± 4.14	85.5 ± 63.3	<2[7]	2	2	E,F
White-beaked dolphin	9	M	Gulf of St. Lawrence	43.4 ± 26.7	46.0 ± 22.1	34.2 ± 22.4	ND	ND	ND	G
Pilot whale	5	M	Newfoundland S. Coast	11.9 ± 6.10	11.7 ± 7.05	9.03 ± 3.80	ND	ND	ND	G
Killer whale	6	M+F	Georgia Str./Van. Is.	50.4 ± 52.3	12.5 ± 14.1	31.4 ± 29.3	<2[8]	19	6	F,H
Harbour porpoise	7	M+F	Georgia Str./Van. Is.	13.0 ± 8.4	5.7 ± 3.0	11.3 ± 6.9	2	20	46	F,H
False killer whale	2	M	Georgia Str./Van. Is.	75.9, 1920	16.6, 89.2	45.2, 33.9	<2, 8	2, 109	2, 23	F,H

[1] P = pooled samples, M= males, F = females.

[2] Total PCB congeners except as indicated.

[3] 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin.

[4] References: A, Norstrom et al. (1990); B, Norstrom et al. (1988);C, Muir et al. (1988a);D, Muir et al. (1991); E, Muir et al. (1990); F, Norstrom and Simon (1990); G, Muir et al. (1988b); H, Muir. Unpublished results 1990.

[5] Not determined.

[6] Results PCDDs and PCDFs for liver, all other for fat samples. Pooled samples were analysed except for St. Lawrence beluga and West coast killer whales.

[7] PCDD/PCDF results based on mean of 10 oil samples (M + F) assuming undetectable levels are one-half of the detection limit.

[8] PCDD/PCDF results are means from 6 killer whale blubber samples with similar assumptions as footnote 7.

from a larger study of PCBs and OC pesticides in tissues of polar bears from 12 management zones in the Canadian Arctic (Norstrom et al. 1988). Polar bears feed almost exclusively on seals, and their distribution is well known, so they are an excellent species for characterizing marine contamination. Levels of most organochlorines were lowest in the high Arctic and highest in Hudson Bay polar bears reflecting the relative isolation of the high Arctic groups from North American sources of contaminants (Table 1).

Addison et al. (1984;1986) have used east coast grey seals and Arctic ringed seals to study temporal trends in PCBs and DDT-group compounds. Care was taken to match the samples in terms of age, sex, and blubber thickness. They found significant declines in total DDT (ΣDDT) but not for PCBs in Grey seals over the period 1974 to 1982. Levels of PCBs declined in Arctic ringed seals over the period 1972 to 1981 while declines of DDT-related compounds were not statistically significant.

There are obvious limitations to the use of marine mammals for determining geographic and temporal trends. Sex and age are important sources of variation (Aguilar 1987). Females are generally less contaminated than males because of excretion of lipophilic organochlorines via lactation. Age of sexual maturity and duration of active parturition has been determined in Dall's porpoise based on the relationships of PCBs and 4,4'-DDE to age (Subramanian et al. 1988). Recalcitrant organochlorines such as hexa- to nonachloro- PCBs, 4,4'-DDE and trans-nonachlor (a component of chlordane) are usually significantly correlated with age in male seals (Addison and Smith 1973; Muir et al. 1988) and toothed and baleen whales (Tanabe et al. 1987; Aguilar and Borrell, 1988).

Age and sex can be taken into account in geographic and temporal comparisons by selecting age classes of the same sex, but factors such as lack of knowledge of diet of the animals, availability of stranded versus hunted animals, and differences in analytical methodology (including technological changes in detection over time) may also influence the conclusions of such studies. In the Canadian Arctic adult male narwhals and belugas from the Davis Strait and Baffin Bay region had significantly higher ΣPCBs, ΣDDT and PCCs than animals of approximately the same age in Hudson Bay and the Beaufort Sea (Table 1). The reasons for this are not known but dietary differences may be important because narwhal are known to prey on Greenland halibut, a deep sea predator, found in Davis Strait but not present in the shallow waters of Hudson Strait or the Beaufort Sea (Muir et al. 1991). St. Lawrence beluga can be distinguished from other beluga stocks and from cetaceans in the Gulf of St. Lawrence by higher levels of mirex (Muir et al. 1990). Although the diet of the beluga is varied, including benthic organisms as well as pelagic invertebrates and fish, levels of mirex in the animals can be accounted for by assuming that eels, which migrate annually from Lake Ontario to the Sargasso Sea, form a small portion (<10%) of the diet (Béland and Martineau 1988).

Although samples from stranded animals are commonly used in studies of contaminants in marine mammals there is always the question of how representative they are of the population. Levels of organochlorines in tissues may vary because of loss of lipid and transformation of the contaminants in the decomposing tissues (Borrell and Aguilar 1990). Bergman et al (1981) found that PCB levels in dead, stranded ringed and Grey seals from the Baltic Sea were higher than in live (hunted) animals and that levels did not correlate with age. Results from the St. Lawrence beluga and west coast killer whales (Table 1) are all from stranded animals and may be subject to these problems. However, St. Lawrence beluga have several characteristics in common with their (hunted) Arctic relatives such as similar blubber lipid levels, lower contaminant levels in females than in males and positive correlations of tissue concentrations in males with age. On the other hand, stranded female beluga from the St. Lawrence are older than those from the Arctic and have positive correlations of PCBs and ΣDDT with age, indicating a lower frequency of parturitions (Muir et al. 1990).

A further complication in interpreting survey of OCs levels in marine mammals, especially between species, is metabolism of the contaminants. The pattern of PCB congeners in odontocetes is similar; penta- and hexachlorobiphenyls predominate and trichlorobiphenyls are virtually absent. This contrasts with most marine fish and invertebrates in which tri- and tetrachlorobiphenyls usually predominate (Duinker and Hillebrand 1983; Muir et al. 1988a). The 2,5-and 2,3,6-substituted tetra-, penta- and hexachloro- congeners are more prominent in cetaceans and pinnipeds than in terrestrial mammals (Tanabe et al. 1988). Polar bears have the most remarkable capability to metabolize PCBs, DDT and chlordane-related compounds. Three congeners, all with 2,4,5- and 2,3,4,5-substitution, accounted for 71% of ΣPCB in polar bear fat (Norstrom et al. 1988).

Marine mammals appear to have a wide range of capabilities to metabolize PCDDs and PCDFs. Arctic ringed seal feeding in the same region as beluga and polar bear had higher levels of 2,3,7,8-TCDD, TCDF and OCDD than the latter species, although they had from 5 to 10-fold lower levels of PCBs (Table 1). Buckland et al. (1990) detected only 2,3,7,8-substituted PCDDs and PCDF congeners in Hector's dolphin from New Zealand, the usual finding for birds, fish and terrestrial mammals. The St. Lawrence beluga had undetectable 2,3,7,8-TCDD but did contain low ng/kg levels of 1,2,4,7,8-PnCDF, 1,2,4,6,7,8-, 1,2,4,6,8,9-HxCDF. Dall's porpoise from Georgia Strait/Vancouver Island also contained the higher chlorinated non-2,3,7,8-substituted PCDFs while killer whales, from the same waters, did not (Norstrom and Simon, unpublished data 1990). The most prominent PCDD in marine mammals from Georgia Strait was 1,2,3,6,7,8-HxCDD (Table 1). The source of this contaminant is probably the use chlorophenols and of chlorophenol-contaminated wood chips in bleached kraft mills.

Studies of the patterns of PCBs and PCDD/PCDF congeners give some insight into marine mammal physiology. Whales have relatively low levels of metabolic activity towards phenobarbital-type substrates, relative to rats, but high levels of activity of isozymes that metabolize PAHs (Watanabe et al. 1989). The consequences of elevated mixed function oxidase activity at the level of individual animals and populations are unknown, however, altered physiological processes, i.e. reproductive failures, have been associated with high levels of PCBs in some marine mammal populations (Helle et al. 1976; Reijnders 1980;Olsson 1986; Martineau et al. 1987).

As top predators with long life spans, marine mammals should be good indicators of ecosystem "health". However direct cause and effect relationships between levels of contaminants and effects such as reproductive failure have been difficult to establish (Reijnders 1986a; Addison 1989). This is a consequence of the lack of information on biochemical and physiological effects of the contaminants in marine mammals, and because of the mixture of pollutants to which the animals are exposed to in their diet. Studies with mink and with captive habour seals have shown reproductive failure can be induced on a fish diet high in PCBs (Reijnders 1986b; Bleavins et al. 1980). Recent studies have identified non-ortho-substituted PCBs as the toxicants inducing reproductive failures in mink (Olsson et al. 1990).

In conclusion, marine mamals are good indicators of geographic and temporal trends of recalcitrant OCs such as highly chlorinated PCB congeners, chlordane- and DDT-related compounds, provided that effects of age, sex and diet are considered. Cetaceans appear to have a wide range of capability to metabolize PCDD/PCDFs and are therefore not good indicators for geographic trends of these components. Marine mammals can also serve as indicators of ecosystem health but direct cause and effect relationships between observed biological effects and contaminant levels remain to be established.

ACKNOWLEDGMENTS

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FISH HEPATIC MONO-OXYGENASES IN MARINE ENVIRONMENTAL QUALITY ASSESSMENT. R.P. Addison. Physical and Chemical Sciences Branch, Department of Fisheries and Oceans. Scotia-Fundy Region, Bedford Institute of Oceanography. Dartmouth, NS Canada B2Y 4A2 (902) 426-3279.

Fish hepatic mono-oxygenases (mixed function oxidases: MFO) are induced by a range of environmental contaminants including some polynuclear aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and chlorinated dibenzodioxins and dibenzofurans. Measurement of MFO activity therefore indicates the presence and effects of such compounds. In this paper, the use of MFO measurements to assess the scale and duration of pollution events, usually involving PAH, is reviewed. The success of MFO measurements in international exercises sponsored by IOC and ICES to evaluate methods of "biological effects monitoring" and the application of MFOs in future large-scale marine environmental quality monitoring programmes will be discussed.

OIL POLLUTION MONITORING - VALUE OF CHEMOMETRICS TO DATA QUALITY AND INTERPRETATION.

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With modern instrumental systems, particularly the "hyphenated" ones such as gas chromatography-mass spectrometry, it is relatively easy to generate a huge data set from a single determination. When tens or even hundreds of samples are analyzed, the researcher is faced with an apparent overload of information. Over the last couple of decades, however, mathematical and statistical techniques have been developed to extract the essential information from such chemical data sets. Indeed, this information can generally be presented in 2D or 3D plots that are easy to comprehend. Another virtue of the methods is that they are very sensitive to bad data points. Reinspection of data prompted by this sensitivity reveals misplaced decimal points, inverted numbers, peak misassignments and the like, thereby greatly enhancing data quality. An ongoing study of hydrocarbons and other compounds in the Mackenzie River and Estuary has successfully used chemometric tools to enhance data quality and understanding. This study will provide a sound basis for measuring the impact of future oil production in the southern Beaufort Sea.

EVALUATION PROCEDURES FOR DREDGED MATERIAL DISPOSAL IN OCEAN WATERS OF THE UNITED STATES

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ABSTRACT

The *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters*, commonly referred to as the "Green Book," contains technical guidance on determining the suitability of dredged material for ocean disposal in U.S. waters. The United States Environmental Protection Agency and the United States Army Corps of Engineers published the original manual in 1977. The revised guidance manual, presently in draft form, is being updated to reflect dredging-program experience and to incorporate the improvements in evaluative testing.

Integral to the revised guidance manual is a tiered testing protocol that incorporates "pass/fail" decision points. The procedure comprises four levels (tiers) of increasing investigative intensity that generate information to assist in making ocean disposal decisions. Tiers I and II utilize existing or easily acquired information and tests that are relatively inexpensive, and apply rapid procedures to determine environmental effects. Tiers III and IV contain biological evaluations that are more intensive and require field sampling, laboratory testing, and rigorous data analysis.

INTRODUCTION

The *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters*, commonly referred to as the "Green Book," contains technical guidance for determining the suitability of dredged material for ocean disposal through chemical, physical, and biological evaluations. This guidance is used by dredging applicants, laboratory scientists, and regulators to evaluate dredged-material compliance with U.S. Ocean Dumping Regulations, Title 40 Code of Federal Regulations Parts 220-228 (40 CFR 220-228). The basis of 40 CFR 220-228 is the Marine Protection, Research, and Sanctuaries Act of 1972, which requires that ocean disposal of dredged material not cause adverse impact to the marine environment.

The United States Environmental Protection Agency (EPA) and the United States Army Corps of Engineers (CE) published the original Green Book in 1977. Since then many advancements have been made in the evaluation methods of dredged material and in understanding the impact of ocean disposal. Also since 1977, region-specific criteria and policies have evolved for dredged-material disposal in U.S. waters, resulting in a wide range of sediment-testing procedures along the Atlantic, Pacific, and Gulf of Mexico coasts. For these reasons, the guidance in the Green Book is now being updated to reflect dredging-program experience, to incorporate improved evaluative testing, and to achieve an environmentally sound level of national regulatory consistency.

In January 1990, EPA and the CE published a draft of the revised 1977 Green Book and distributed it to Federal, State, and local regulatory personnel, port authorities, and other individuals and companies involved in dredged-material evaluation. In the subsequent months, EPA and the CE have conducted training sessions on the new guidance and solicited public and Agency comments on the new manual. The manual is now undergoing minor revisions to address the comments received by EPA and the CE. Finalization and promulgation of the manual is expected in early 1991. Until this date, the guidance of the 1977 manual is still in force.

Concurrent with the work on the Green Book, EPA is revising the Ocean Dumping Regulations to improve their clarity, reflect dredging-program experience, and to incorporate various statutory changes. The 1990 Green Book will be modified as needed to correlate with the revised regulations.

This paper introduces the technical components of the 1990 Green Book. Integral to the manual is a tiered-testing protocol to characterize dredged material and predict its impact on the water column and the benthos at ocean disposal sites. This protocol was developed out of consensus among EPA and CE personnel and testing-laboratory researchers, and it balances the requirements of the Ocean Dumping Regulations, state-of-the-art dredged-material evaluation techniques, and the realities of the testing and permitting process for new and existing projects. Local expertise is both recommended and necessary to adapt the National guidance in the manual to specific dredged-material projects. Three EPA Regions and CE Districts have begun to apply the National guidance of the 1990 manual through the development of regional guidance manuals. In summary, the Green Book

- Provides for national consistency in evaluating dredged material for ocean disposal
- Ensures adherence to the Ocean Dumping Regulations
- Incorporates existing (and valuable) regional expertise and guidance in the evaluation process

Tiered Testing

The tiered-testing protocol in the Green Book comprises four procedural tiers, with decision points at each tier (Figure 1) to assist in decision-making for dredged-material disposal. Each successive tier provides increasing investigative intensity to generate the information for permitting decisions on ocean disposal.

- Tier I primarily assesses existing information on the proposed dredged material and identifies the contaminants of concern.
- Tier II uses calculations and numerical models to screen the chemical and physical characteristics of the dredged material and the overall conditions at the disposal site.

- Tier III consists of standardized acute bioassays and bioaccumulation tests on laboratory organisms.
- Tier IV tests specific projects for the results of long-term organism exposure to the dredged material that may influence reproduction and species survival.

The methods and evaluative strategies in each of the tiers are recommended to achieve National technical consistency and increased intersite comparability of data sets and analyses. The principal purpose of the tiered-testing protocol is to determine if the limiting permissible concentration (LPC)* is met as defined in Section 227.13(c) of the Ocean Dumping Regulations.

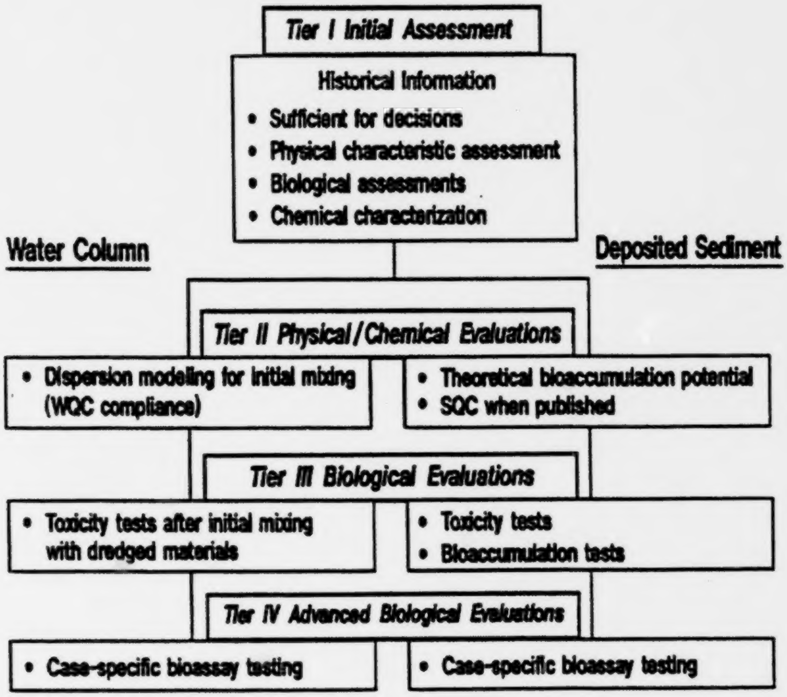
Tier I Initial Assessment
Historical Information
• Sufficient for decisions
• Physical characteristic assessment
• Biological assessments
• Chemical characterization
Water Column
Deposited Sediment
Tier II Physical/Chemical Evaluations
• Dispersion modeling for initial mixing (WQC compliance)
• Theoretical bioaccumulation potential
• SQC when published
Tier III Biological Evaluations
• Toxicity tests after initial mixing with dredged materials
• Toxicity tests
• Bioaccumulation tests
Tier IV Advanced Biological Evaluations
• Case-specific bioassay testing
• Case-specific bioassay testing

Figure 1. Overview of Tiered-Testing Protocol for Evaluating Dredged Material

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*LPC of the water column is defined in the Green Book as the concentration of dredged material that, after allowance for initial mixing, does not exceed applicable marine water-quality criteria or a toxicity threshold of 0.01 of the acutely toxic concentration. The LPC of the suspended particulate and solid phases is defined as that which will not cause unreasonable toxicity or bioaccumulation.

"Green-light," "yellow-light," or "red-light" LPC evaluations are reached as the dredged-material evaluator proceeds through the tiers.

- Green Light
  The LPC is met and the ocean disposal option is supported.

- Yellow Light
  The LPC evaluation is inconclusive; proceed to the next tier

- Red Light
  The LPC is not met and the ocean disposal option is not supported.

The green-light for *both* water-column and benthic LPC evaluations must be reached for consideration of the ocean disposal option to proceed. A yellow-light evaluation in Tiers I-III requires the dredging applicant to conduct additional testing in subsequent tiers or to decide to not ocean-dump. However, a red-light evaluation does not necessarily exclude all possibilities for ocean dumping. For instance, if appropriate management actions can make the dredged material meet the LPC, ocean dumping could be allowed. Management-action procedures such as disposal-site capping, reducing the rate of disposal, treating the dredged material to immobilize or transform contaminants, or other alternatives, could be considered. Management actions for red-light evaluations are *not* included in the Green Book because of the wide range of available options and the project-specific nature of such work.

The tiered-testing protocol is relatively flexible. As presently written, the dredged-material evaluator can enter and exit the dredged-material testing procedures at any tier. However, to begin the evaluation in Tier II, III, or IV, the existing data must satisfy the requirements of the earlier tier(s). To exit a tier before reaching a green light requires the dredging applicant to select non open-ocean disposal.

Dredged material that cannot be definitively evaluated under Tiers I, II, and III must be evaluated under Tier IV. In such cases, the applicant might choose to not spend additional time and resources on Tier IV testing and instead select a non open-ocean disposal alternative. Similarly, an applicant can try to save time and money by proceeding directly to Tier II, III, or IV if it is believed that analysis in the earlier tiers will not lead to a definitive evaluation. The applicant can also choose to continue testing under later tiers to support an evaluation reached in an earlier tier. The only absolute requirement is that the dredged material must comply with the regulations if it is to be dumped in the ocean. The tiered-testing protocol facilitates this determination.

Tier I: Initial Assessments

The purpose of Tier I (Figure 2) is to identify contaminants of concern and determine dredged-material compliance through analysis of existing chemical, physical, and biological information. For a green light to be reached in this tier, the information must be sufficient to conclude that the material is in full compliance with the LPC. For many dredging operations, there is a wealth of readily available information on the proposed dredged material and on the characteristics of the disposal site. This is especially true of areas that have historically undergone maintenance dredging or have been the subject of other studies, such as fish-stock assessments. The available information for a given area might not be sufficient to reach a final LPC evaluation, but often there are accessible high-quality data that can supplement the results of tests in subsequent tiers and facilitate reaching an early decision with lowered expenditure of resources. Table 1 lists the possible sources of information that can be used to partially or fully evaluate proposed dredging operations. The list is not intended to be comprehensive. Other sources may be considered for additional information. Whatever the source of information for Tier I, the quality of the data must be evaluated and weighed accordingly. The references in Chapter 13 of the manual, Quality Assurance (QA) Considerations, can be consulted for guidance for evaluating the quality of data obtained from different information sources.

If the information is sufficient to determine water-column and deposited-sediment effects in Tier I, either a red light or green light is reached: (1) The LPC is not met and the ocean-disposal option is not supported. (2) The LPC is met and the ocean-disposal option is supported (if all other requirements of the regulations are satisfied). An evaluation at this tier usually requires expert analysis of the information on the characteristics of both the proposed dredged material and of the environment of the disposal site. If the information is not sufficient to reach a decision within this tier (yellow light), the evaluative process moves into Tier II.

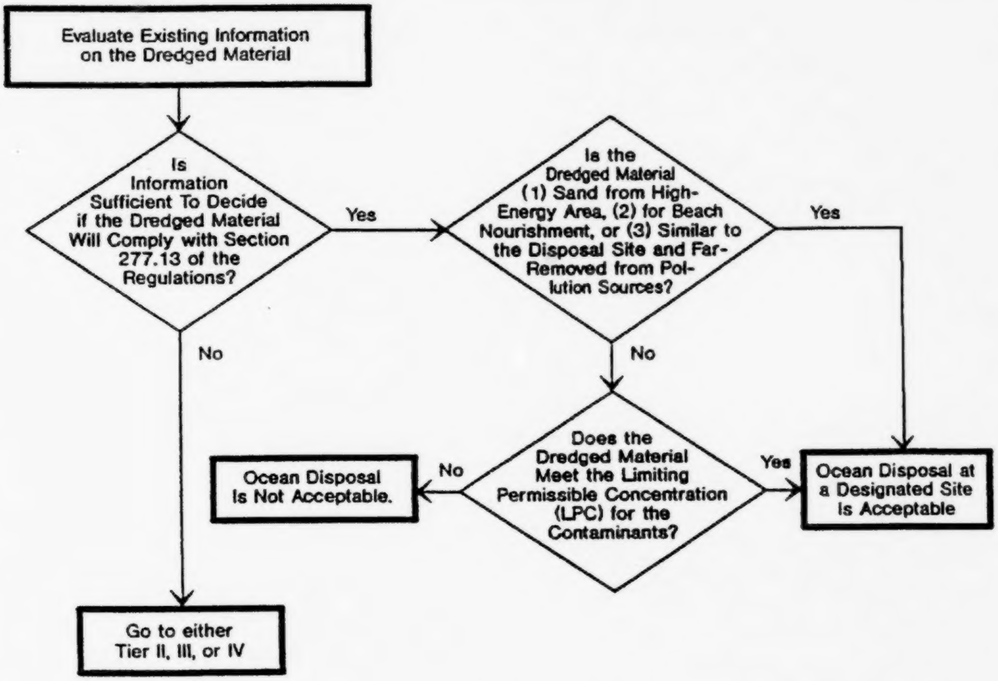
Evaluate Existing Information on the Dredged Material
Is Information Sufficient To Decide if the Dredged Material Will Comply with Section 277.13 of the Regulations?
Yes
No
Is the Dredged Material (1) Sand from High-Energy Area, (2) for Beach Nourishment, or (3) Similar to the Disposal Site and Far-Removed from Pollution Sources?
Yes
No
Does the Dredged Material Meet the Limiting Permissible Concentration (LPC) for the Contaminants?
No
Yes
Ocean Disposal Is Not Acceptable.
Ocean Disposal at a Designated Site Is Acceptable
Go to either Tier II, III, or IV

Figure 2. Tier I: Evaluation of Existing Information

Table 1: 1990 Green Book Information Sources for Tier I Dredged-Material Evaluation

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- Study reports on prior chemical, physical, or biological tests on the material proposed to be dumped or on similar materials
- Study reports on prior environmental monitoring on the material proposed to be dumped or on similar materials
- Local public and private records on potential contaminants of concern entering the proposed dredged-material sediments
- Selected Chemical Spill Listing records (EPA)
- Pesticide Spill Reporting System records (EPA)
- Pollution Incident Reporting System records (United States Coast Guard)
- Identification of in-place pollutants and priorities for removal (EPA)
- Hazardous-wastes sites and management facilities reports (EPA)
- CE studies of sediment pollution and sediments
- STORET, BIOS, CETIS, and ODES databases (EPA)
- Water and sediment data on major tributaries [U.S. (Geological Survey)]
- National Pollutant Discharge Elimination System (NPDES) permit records
- Section 404(b)(1) evaluations
- Pertinent and applicable research reports
- Section 103 evaluations
- Port Authorities' records
- College and university published/unpublished information
- Records of State environmental agencies
- Published scientific literature

Tier II: Physical/Chemical Evaluations

Under Tier II, water-column and benthic evaluations are made separately. The purpose of this tier is to provide reliable, rapid, environmentally conservative screening for potential impact. This is possible to achieve for water-column evaluations by using a numerical mixing model. At present, there are no approved methods to comprehensively evaluate deposited sediment at this tier. Only nonpolar organic compounds in sediment can be evaluated under Tier II at this time. When sediment-quality criteria (SQC) are promulgated, they will be incorporated into Tier II.

Tier II: Water-Column Physical/Chemical Evaluations

Tier II water-column evaluations use information acquired in Tier I (Figure 3). If water-quality criteria (WQC) are unavailable for all of the contaminants of concern in the proposed dredged material or synergistic effects among the contaminants are suspect, testing must be performed in Tier III. (Synergism is usually suspected if more than one contaminant is present.) However, if WQC are available for the contaminants and no synergism is suspected, a red-light/green-light water-column evaluation can be reached at this tier through the application of a numerical mixing model.

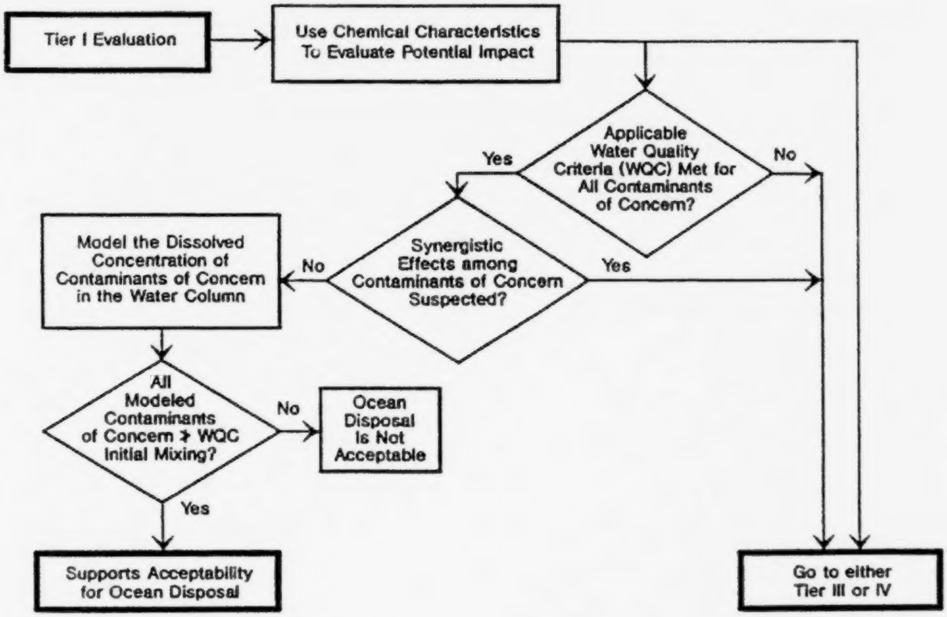
Tier I Evaluation
Use Chemical Characteristics To Evaluate Potential Impact
Applicable Water Quality Criteria (WQC) Met for All Contaminants of Concern?
Yes
No
Synergistic Effects among Contaminants of Concern Suspected?
No
Yes
Model the Dissolved Concentration of Contaminants of Concern in the Water Column
All Modeled Contaminants of Concern ≯ WQC Initial Mixing?
No
Ocean Disposal Is Not Acceptable
Yes
Supports Acceptability for Ocean Disposal
Go to either Tier III or IV

Figure 3. Tier II: Water-Column Physical/Chemical Evaluations

Numerical Models for Initial Mixing

The numerical models in the Green Book evaluate dredged-material dilution during the initial-mixing phase of ocean disposal. Section 227.29 of the regulations defines initial mixing as 4 hours following a dredged-material dump. During this 4-hour period, the concentration of the contaminants in the water column is allowed to exceed the LPC *within the boundary of the disposal site.* However, if water currents transport the settling dredged material out of the disposal site before 4 hours expires, the point in time when the material crosses the site boundary is used in determining compliance. Exceeding the LPC outside the site *at any time* is a violation of the regulations.

The Automated Dredging and Disposal Alternatives Management System (ADDAMS) models, developed by the CE, are the recommended models for evaluating initial mixing of dredged material at ocean disposal sites. ADDAMS models can be run on a personal computer with a minimum of hardware. The models account for the physical processes of dredged-material disposal at open-water disposal sites by calculating water-column concentrations of dissolved contaminants and suspended sediments and the initial deposition of material on the bottom. Three separate ADDAMS models address different methods of disposal:

• DIFID	Disposal from an instantaneous dump
• DIFCD	Disposal from a continuous discharge
• DIFHD	Disposal from a hopper dredge

To evaluate initial mixing following ocean disposal, the appropriate model is run *for the contaminant requiring the greatest amount of dilution* to meet the LPC. The models simulate movement of the disposed material as it falls through the water column, as it is transported and diffused by the ambient current, and as it spreads over the bottom. The models have some limitations, e.g., the DIFID model will not work for very shallow disposal sites where the discharge time from the barge exceeds the descent period to the bottom. However, the models can simulate a wide range of disposal options. EPA and the CE are in the process of field-verifying these models. When the models are fully verified and approved, they will be able to support definitive water-column evaluations and, thereby, reduce additional time and expense of running Tiers III and IV evaluations.

The models treat the descending dredged material as a dense liquid. This assumes that all of the constituents in the material are released into the water column and that the LPC can be evaluated in a conservative manner. At a typical disposal site, unless it is extremely deep (>300 m), the dredged material usually settles, with its contaminants, to the bottom in clumps.

The Green Book contains an appendix on the ADDAMS models and an early 1990 version of the programs on computer diskettes. Since distribution of the 1990 manual, the models have been revised to be more user friendly and CE modelling personnel are available at the CE Waterways Experiment Station (WES), Vicksburg, Mississippi, to supply the latest versions of the models, answer questions, and assist with running the appropriate models. In general, model input parameters include

- Disposal-site descriptions
- Disposal-operation descriptions
- Disposal-site water-current velocity descriptions
- Dredged-material descriptions
- Coefficients for the movement of the dredged material through the water column
- Input, output, and execution descriptions.

Model output includes

- Repetition of the input data for QA considerations
- History of the descent and collapse phases of the discharge in both numeric and diagrammatic displays.

In DIFID and DIFHD, the following time-dependent information can be requested.

- Size of the collapsing cloud of dredged material in the water column
- Cloud density
- Centroid location and velocity
- Contaminant and solids concentration

The model output can present water-column contaminant concentrations in milligrams per liter. These concentrations are compared to the appropriate LPCs to determine compliance at the boundary of the disposal site or compliance within the site following the 4-hour initial-mixing period.

Tier II: Benthic Physical/Chemical Evaluations

As discussed above, presently only benthic effects attributed to nonpolar organic chemicals in the deposited sediment can be addressed in Tier II (Figure 4). Nonpolar organic chemicals include all organic compounds that do not dissociate or form ions. This includes chlorinated hydrocarbon pesticides, other halogenated hydrocarbons, polychlorinated biphenyls, most polynuclear aromatic hydrocarbons, dioxins, and furan. It does not include polar organic compounds, organometals, and metals. If all of the contaminants of concern in the dredged material are *nonpolar* organic compounds, the theoretical bioaccumulation potential (TBP) can be calculated for the dredged material and a reference sediment* to determine LPC bioaccumulation compliance at this tier. The TBP calculation is based on concentration of the nonpolar organic chemicals in the sediment, the total organic carbon concentration, and the percent lipid content of an organism of interest. If the TBP of the dredged material is not statistically greater than that of the reference material, then a green light is reached for bioaccumulation evaluation under Tier II. (Acute-toxicity evaluations must be performed under Tier III unless sufficient toxicity information had been obtained under Tier I.)

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*A reference sediment is defined as a sediment, substantially free of contaminants, that has grain-size characteristics as similar as practicable to the dredged material and to the sediment at the disposal site, and that reflects conditions at the disposal site as though dredged-material disposal had taken place.

If any of the contaminants of concern are polar organic compounds or have suspected toxic components or the dredged-material TBP exceeds the reference material TBP described above, the evaluation for benthic impact by the dredged material must take place in Tier III or IV. At present, only a green-light or a yellow-light outcome for bioaccumulation evaluation is possible under Tier II. The need for additional tests in Tier II to screen for benthic impact is recognized by EPA and the CE, and new tests are under development and evaluation. When the scientific and regulatory community verifies one or more of these tests, they will be incorporated into Tier II in a future Green Book revision. In the meanwhile, evaluation of benthic impact that cannot be made in Tier I must be completed in Tier III or IV.

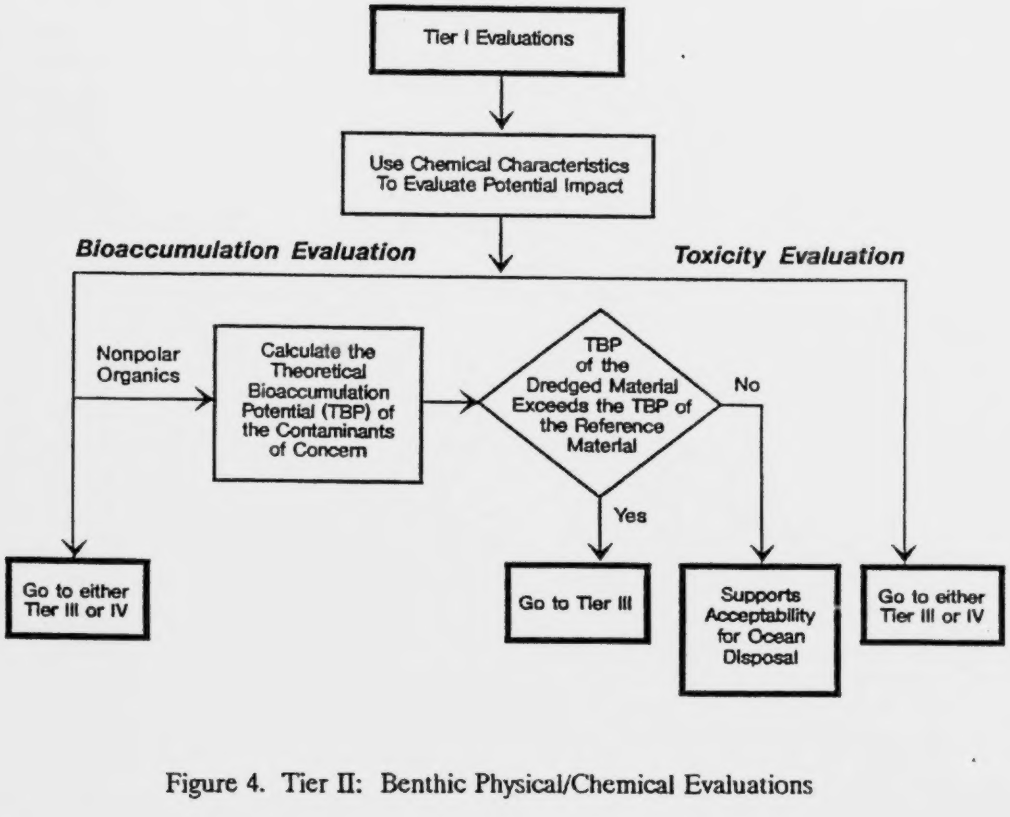
Tier I Evaluations
Use Chemical Characteristics To Evaluate Potential Impact
Bioaccumulation Evaluation
Toxicity Evaluation
Nonpolar Organics
Calculate the Theoretical Bioaccumulation Potential (TBP) of the Contaminants of Concern
TBP of the Dredged Material Exceeds the TBP of the Reference Material
No
Yes
Go to either Tier III or IV
Go to Tier III
Supports Acceptability for Ocean Disposal
Go to either Tier III or IV

Figure 4. Tier II: Benthic Physical/Chemical Evaluations

Tier III: Biological Evaluations

Tier III testing includes (1) determination of water-column toxicity according to the regulatorily defined suspended phase and (2) an assessment of contaminant toxicity and bioaccumulation from the material to be dredged. The evaluations in this tier are based on the output from Tiers I and II and comprise standardized bioassays with the organisms listed in Table 2.

Tier III: Water-Column Biological Evaluations

Tier III water-column tests are acute tests that evaluate the toxicity of the dissolved and suspended portions of the dredged material that remains in the water column after initial mixing (i.e., 4 hours postdisposal) (Figure 5). The bioassays are run if the Tier II evaluations are inconclusive, i.e, there are not applicable WQC for all contaminants of concern or there is reason to suspect synergistic effects among the contaminants. (See Tier II.) Tier III involves exposing fish, crustaceans, and zooplankton to a dilution series containing both dissolved- and suspended-sediment components of the dredged material. A typical test monitors organism mortality over a 96-hour period.

The results of the bioassays are used to calculate the LC_{50} concentration of the dredged material in the water column. The LPC for this evaluation is 1% of the LC_{50}. Following the determination of the LPC, a red-light or green-light evaluation is reached with the application of the numerical model (discussed above).

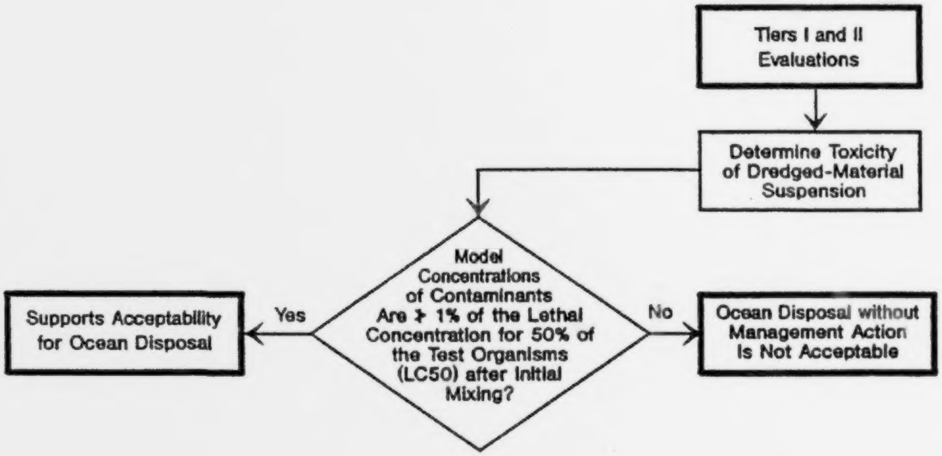
Tiers I and II Evaluations
Determine Toxicity of Dredged-Material Suspension
Model Concentrations of Contaminants Are ≯ 1% of the Lethal Concentration for 50% of the Test Organisms (LC50) after Initial Mixing?
Yes
Supports Acceptability for Ocean Disposal
No
Ocean Disposal without Management Action Is Not Acceptable

Figure 5. Tier III: Water-Column Biological Evaluations

Table 2. Species for Water-Column and Benthic Evaluations in the 1990 Green Book

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Water Column Species

- Crustaceans
  - Mysids
    - *Mysidopsis* sp.[a]
    - *Neomysis* sp.[a]
    - *Holmesimysis* sp.[a]
  - Shrimp
    - *Palaemonetes* sp.
    - *Penaeus* sp.
    - *Pandalus* sp.
  - Crab
    - *Callinectes sapidus*
    - *Cancer* sp.
- Fish
  - *Menidia* sp.[a]
  - *Cymatogaster aggregata*[a]
  - *Lagodon rhomboides*
  - *Leiostomus xanthurus*
- Zooplankton
  - Copepods
    - *Acartia* sp.[a]
  - Mussel larvae
    - *Mytilus edulis*[a]
  - Oyster larvae
    - *Crassostrea virginica*[a]
    - *Ostrea* sp.[a]
  - Crustacean larvae
    - Recommended species[a]

Benthic Species

- Crustaceans
  - Infaunal Amphipods
    - *Rhepoxynius* sp.[a]
    - *Ampelisca* sp.[a]
    - *Eohaustorius* sp.[a]
  - Mysids
    - *Mysidopsis* sp.
    - *Neomysis* sp.
    - *Holmesimysis* sp.
  - Shrimp
    - *Penaeus* sp.
    - *Palaemonetes* sp.
    - *Crangon* sp.
    - *Pandalus* sp.
  - Crab
    - *Callinectes sapidus*
    - *Cancer* sp.
- Burrowing Polychaetes
  - *Neanthes* sp.[a]
  - *Nereis* sp.[a]
  - *Nephthys* sp.
  - *Glycera* sp.
  - *Arenicola* sp.
  - *Abarenicola* sp.
- Molluscs
  - *Yoldia limatula*
  - *Macoma* sp.

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[a] Recommended test species

Tier III: Benthic Biological Evaluations

Benthic evaluations in Tier III consist of toxicity and bioaccumulation tests with the organisms that are listed in the righthand column of Table 2 (Figure 6). To conduct these test, the Green Book provides laboratory guidance on sediment preparation, reference- and control-sediment tests, treatment replicates, organism handling, test-chamber conditions, QA/QC considerations, and data analysis. The organisms used in the tests are surrogates for disposal species and are used to estimate dredged-material effects. The toxicity tests quantify lethality. If the mortality in the dredged-material bioassays is greater than 10%* over the mortality in the reference-sediment bioassays, the LPC are not met (red light). If, however, acute toxicity in the dredged-material tests is less than 10% above that in the reference-sediment tests, the LPC is met (green light).

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*Some approved tests allow for a larger percentage.

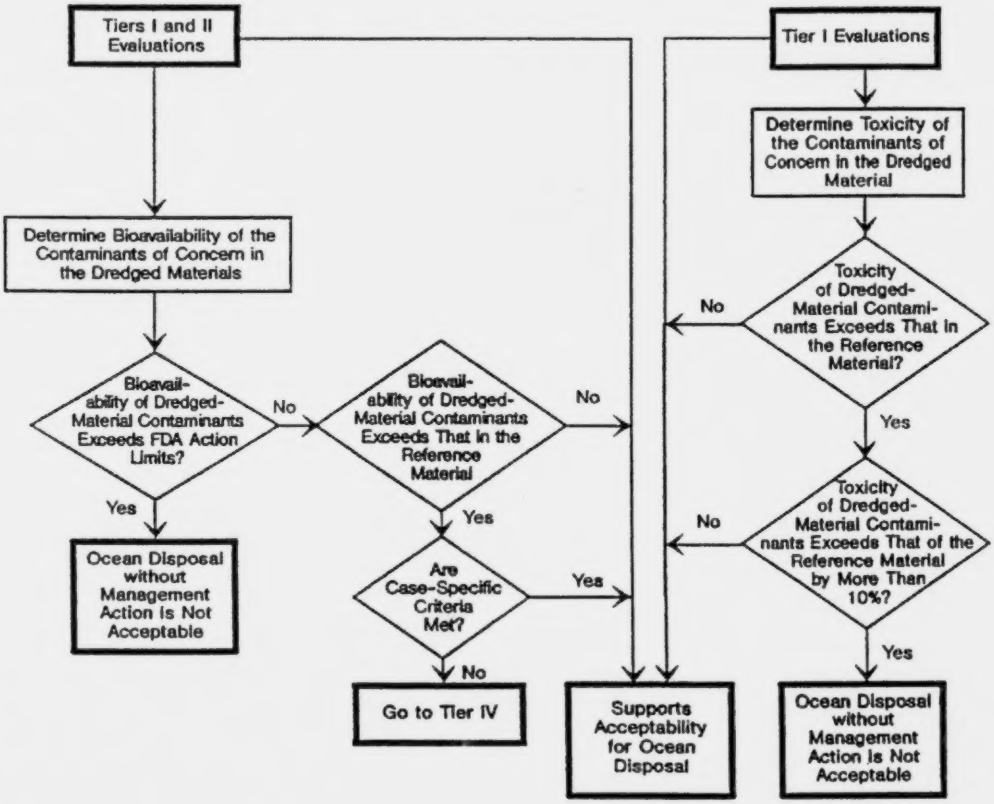
Tiers I and II Evaluations
Determine Bioavailability of the Contaminants of Concern in the Dredged Materials
Bioavailability of Dredged-Material Contaminants Exceeds FDA Action Limits?
No
Yes
Ocean Disposal without Management Action Is Not Acceptable
Bioavailability of Dredged-Material Contaminants Exceeds That in the Reference Material
No
Yes
Are Case-Specific Criteria Met?
Yes
No
Go to Tier IV
Supports Acceptability for Ocean Disposal
Tier I Evaluations
Determine Toxicity of the Contaminants of Concern in the Dredged Material
Toxicity of Dredged-Material Contaminants Exceeds That in the Reference Material?
No
Yes
Toxicity of Dredged-Material Contaminants Exceeds That of the Reference Material by More Than 10%?
No
Yes
Ocean Disposal without Management Action Is Not Acceptable

Figure 6. Tier III: Benthic Biological Evaluations

The bioaccumulation tests (usually run concurrently with the toxicity tests) evaluate the potential of benthic organisms to accumulate contaminants from the dredged material in their tissues. At the conclusion of the tests, the tissue of the organisms are analyzed for the contaminants of concern that were identified in Tier I. Extrapolation of the bioaccumulation-test results is used to assess potential transfer of contaminants into the marine food web.

Section 227.27 of the regulations requires that benthic bioassays be conducted on dredged material with filter-feeding, deposit-feeding, and burrowing species. Infaunal amphipods, such as *Ampelisca* sp. and *Rhepoxynius* sp., are strongly recommended in the Green Book as the preferred species for toxicity tests. They are sensitive bioindicators of impact as they both filter and deposit feed and they build burrowing tubes in benthic sediments. For bioaccumulation evaluations, the Green Book recommends using a burrowing polychaete (e.g., *Neanthes* sp. or *Nereis* sp.) and a deposit-feeding bivalve mollusc (e.g., *Macoma* sp. or *Yoldia limatula*). In summary, the manual recommends that two species be tested for acute toxicity and two additional species for bioaccumulation evaluation. Each set of test species should cover the three species types stipulated in the regulations. The ecological and economic relevance of the organisms and the practical aspects of using the species in the laboratory, such as tolerance to grain-size ranges and year-round availability, also must be considered when selecting the test species.

The Tier III bioaccumulation evaluation compares the contaminant level in the tissues of the organisms to two criteria: (1) the United States Food and Drug Administration (FDA) Action Levels for Poisonous or Deleterious Substances in Fish and Shellfish for Human Consumption and (2) the contaminant levels in the reference-material organisms. Regardless of the statistical comparison to the reference-material test organisms, if the level in the tissues of dredged-material organisms exceeds the FDA levels in any category, the LPC is not met. If the dredged-material results are lower than the FDA action levels and not statistically greater than the reference material, the LPC is satisfied and the ocean-disposal option is supported. However, if bioaccumulation of some contaminants in some species exceeds that found in the reference-material tests, the test results must be evaluated against case-specific criteria. EPA and the CE develop the evaluative criteria case by case from local technical information that addresses the bioaccumulation aspects of the benthic criteria of Section 227.13(c)(3) of the regulations. The purpose of this case-specific bioaccumulation evaluation in Tier III is to reach an environmentally sound red-light or green-light evaluation without having to commit significant time and resources under Tier IV testing.

At present, tests for chronic sublethal exposure to benthic contaminants are being developed. When the tests are approved by EPA, they will be incorporated in Tier III in future revisions to the Green Book.

Tier IV: Advanced Biological Evaluations

Tier IV consists of bioassay and bioaccumulation tests to evaluate the long-term benthic and water-column impact of dredged material (Figures 7 and 8). Tests at this level are selected to

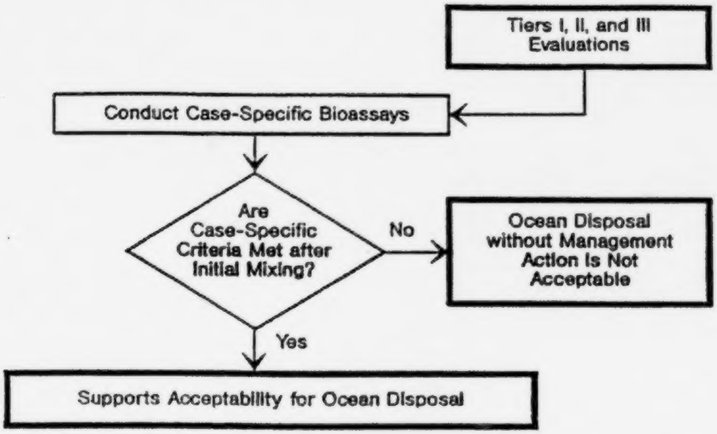
Tiers I, II, and III Evaluations
Conduct Case-Specific Bioassays
Are Case-Specific Criteria Met after Initial Mixing?
No
Ocean Disposal without Management Action Is Not Acceptable
Yes
Supports Acceptability for Ocean Disposal

Figure 7. Tier IV: Advanced Water-Column Biological Evaluations

address specific issues for a specific dredging operation that could not be fully evaluated in the earlier tiers. Since these tests are case-specific and since they require significant time and money to complete, evaluative criteria must be agreed on in advance by EPA and by the CE to determine compliance with the regulations.

Tier IV bioassays help to interpret the bioaccumulation results from Tier III and to measure indicators of long-term effects of clear ecological importance, such as survival and reproduction. The bioaccumulation testing measures the steady-state body burden of contaminants of concern in the tissues of organisms that have been subjected to long-term laboratory exposures or in tissues of appropriately sampled field organisms. The actual contaminant concentrations in the tissues of dredged-material organisms is then compared to the FDA Action Limits and to those of the reference-material organisms, as in Tier III. If the concentrations in the dredged-material organisms are less than the FDA limits but are greater than in the reference-material organisms, they are compared to field-collected organisms from the area of the proposed disposal site. Bioaccumulation levels that exceed those of the disposal-site organisms – but still do not exceed the FDA action levels – are then assessed against case-specific criteria for a final decision on LPC compliance.

In practice, Tier IV testing will seldom be conducted for water-column evaluations because the potential for high water-column or benthic impact will probably become apparent early in the evaluation process and show that the LPC cannot be met. Tier IV benthic testing is more likely, but the Green Book emphasizes that this tier is not intended for routine application. Tier IV benthic tests consume significant resources of the dredging applicant and of the regulatory authority, and a noncompliance evaluation is still possible. The applicant must weigh the options and decide whether to perform Tier IV testing or to consider an alternative such as upland disposal. If the applicant elects to proceed with Tier IV testing, the role of the regulatory authority is to design tests that lead to a definitive LPC evaluation for the project.

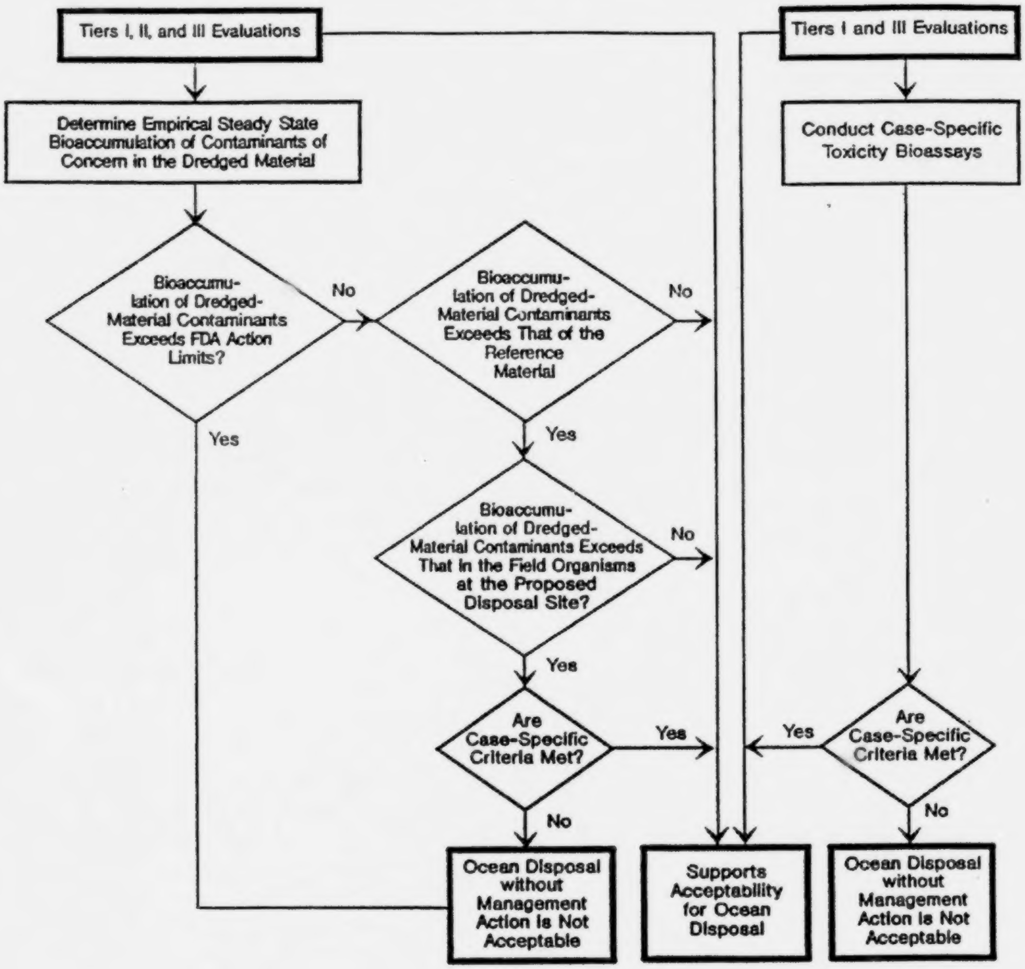
Tiers I, II, and III Evaluations
Determine Empirical Steady State Bioaccumulation of Contaminants of Concern in the Dredged Material
Bioaccumulation of Dredged-Material Contaminants Exceeds FDA Action Limits?
No
Yes
Bioaccumulation of Dredged-Material Contaminants Exceeds That of the Reference Material
No
Yes
Bioaccumulation of Dredged-Material Contaminants Exceeds That in the Field Organisms at the Proposed Disposal Site?
No
Yes
Are Case-Specific Criteria Met?
Yes
No
Ocean Disposal without Management Action Is Not Acceptable
Supports Acceptability for Ocean Disposal
Tiers I and III Evaluations
Conduct Case-Specific Toxicity Bioassays
Are Case-Specific Criteria Met?
Yes
No
Ocean Disposal without Management Action Is Not Acceptable

Figure 8. Tier IV: Advanced Benthic Biological Evaluations

OTHER INFORMATION IN THE MANUAL

In addition to the detailed guidance provided on testing and decision-making within the tiers, the manual also includes sections on sample collection, analytical methods, statistical methods, QA, and a copy of the Ocean Dumping Regulations. The statistics section details the appropriate methods for analyzing bioassay and bioaccumulation data, including sample-size determinations, data-scale transformations, variance homogeneity tests, two-way t tests, analysis of variance (ANOVA), multiple comparisons among treatment means, and confidence interval calculations. The QA section details the importance of QA as a management tool for government regulators and testing laboratories to ensure that the data produced are of known and documented quality.

SUMMARY

The 1990 Green Book is a national guide for dredging applicants, scientists, and regulators to follow in determining if a particular dredged material meets the LPC in the regulations. It is neither a "cookbook" or a comprehensive document. Additional assessments, such as on the economic necessity, related impacts, and analysis of other disposal options, are required before a final permitting decision is reached on ocean disposal. The guidance in the manual must be applied with a thorough understanding of the ocean-dumping regulations and with assistance from the many references cited in the text. The tiered-testing protocol is intended to assist in ecologically sound and efficient decision-making for ocean disposal of dredged material. As new methods and technologies are developed to test dredged material, they will be incorporated into subsequent revisions of the manual.

As the Federal authorities finalize the Green Book, EPA Regions and CE Districts will continue to develop regional companion manuals. These regional manuals will supplement the national guidance in the Green Book and assist applicants and evaluators with permit application and the logistics of project-specific dredged-material evaluation. When the Ocean Dumping Regulations are revised, the guidance in the Green Book will be updated accordingly.

REFERENCES

Environmental Protection Agency/United States Army Corps of Engineers Technical Committee on Criteria for Dredged and Filled Material. 1977. *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters; Implementation Manual for Section 103 of Public Law 92-532 (Marine Protection, Research, and Sanctuaries Act of 1971).* July 1977 (second printing April 1978). Environmental Effects Laboratory, United States Army Engineer Waterways Experiment Station, Vicksburg, MS.

Environmental Protection Agency/United States Army Corps of Engineers. 1990. *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters.* January 1990. United States Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, DC 20460. EPA-503-8-90/002.

PLATFORM SESSION

Sediment Assays

Chair: J. Metcalfe-Smith

The biological significance of contaminants in sediment from Hamilton Harbour, Lake Ontario

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Abstract

Sediment from regions within Hamilton Harbour is highly contaminated with metals and PAHs, nevertheless, not all contaminated sites were highly toxic to test organisms. Most sediment did elicit sublethal and/or lethal responses in bioassay organisms. Results of analyses of tissue residues in test organisms and the amelioration of toxicity by chemical treatment implicate trace metals as contributing to sediment toxicity. Sediment oxygen demand, however, apparently contributed to the restricted benthic community *in situ* and some of the toxicity observed *in vitro*. For some stations, there was evidence that PAHs were responsible for the deleterious effects detected. The suitability for colonization by benthic invertebrates of sediment in some areas of Hamilton Harbour may be limited by both contaminants and high sediment oxygen demand. Remedial options aimed at improving the oxygen regime of the harbour should result in improvements in the benthic invertebrate community directly, by providing a suitable oxygen regime for organisms less tolerant of temporal anoxia, and indirectly by decreasing metal bioavailability, possibly through the coprecipitation of trace metals with iron hydroxides.

Key words: sediment bioassay, sediment toxicity, bioavailability, Hexagenia, Pimephales, metals, PAHs

Introduction

Trace metals and organic compounds in a substantial area of the sediment of Hamilton Harbour exceed the Ministry of Ontario draft sediment guidelines which identify the "severe effects level" (Persaud et al 1990). This is the level at which significant biological impacts are anticipated. These concentrations for total PAH, Cr, Cu, Pb and Zn are 550 (normalized for sediment TOC of 5%), 111, 114, 250 and 800, respectively. Concentrations in the harbour reach a maximum of approximately 700 (TOC 5%), 500, 160, 700 and 4500 for PAH, Cr, Cu, Pb, and Zn, respectively (Rodgers et al 1989). From this one would anticipate significant environmental damage and a necessity to develop remedial options aimed at removing the toxicological threat.

Chemical measurements, however, have been shown to be limited in their use for predicting environmental effects due in large part to the biotic and abiotic factors that mediate metal bioavailability and toxicity. Numerous studies have demonstrated that the geochemistry of a particular system is important in metal speciation (Luoma 1983, Tessier et al 1984, Morse et al 1987, Davis-Colley et al 1985, Campbell et al 1987, Krantzberg and Stokes 1988). This has led to recommendations that biological tests be performed when chemical measurements indicate the potential for adverse environmental impact (Chapman 1989, Landner 1988, International Joint Commission 1988, van Veen and Stortelder 1988, Karr 1987, Persaud et al 1990).

Due to the large volume of contaminated sediment in the harbour, it is unpractical to recommend dredging and disposal of all sediment that exceed the draft provincial guidelines that identify the "severe effects level". It would be extremely useful to be able to determine the extent to which contaminants are biologically available and are having repercussions for the health of the biota.

The principle study objectives were to establish whether contaminants in the harbour are biologically available, to compare the biological response of test organisms to harbour sediment with sediment chemistry, to relate toxicity observed in bioassays to benthic community structure *in situ*, and to evaluate tissue residues of contaminants in test organisms in light of sediment contamination.

A preliminary evaluation of the source of toxicity was investigated by selectively treating sediment with compounds designed to immobilize polar compounds, and comparing the results of bioassays using treated and untreated sediment.

Materials and methods

Sediment collection

In phase one of the study, sediment was collected by Ekman grab from five stations in the harbour and one station situated in Lake Ontario approximately 1 km northeast of the mouth of the harbour (Burlington Ship Canal) in December 1988 (Figure 1). Each station was sampled on two separate occasions during phase one in order to provide information on the variability introduced as a consequence of station relocation. Station numbers are denoted with 1 or 2, thereby indicating on which visit the samples were collected. Phase two, the toxicity evaluation experiment, required the collection of bulk sediment for chemical treatment. Stations were visited once during this phase of the study. For both phases, the surface 2 cm were removed from each grab using acid-washed polyethylene or glass beakers or plastic spoons. Approximately 20 L of surficial sediment was placed in plastic lined collection buckets which were then sealed, kept cold in the field, and stored for no more than two weeks (phase one), or treated and then stored at 4 C for six weeks (phase two).

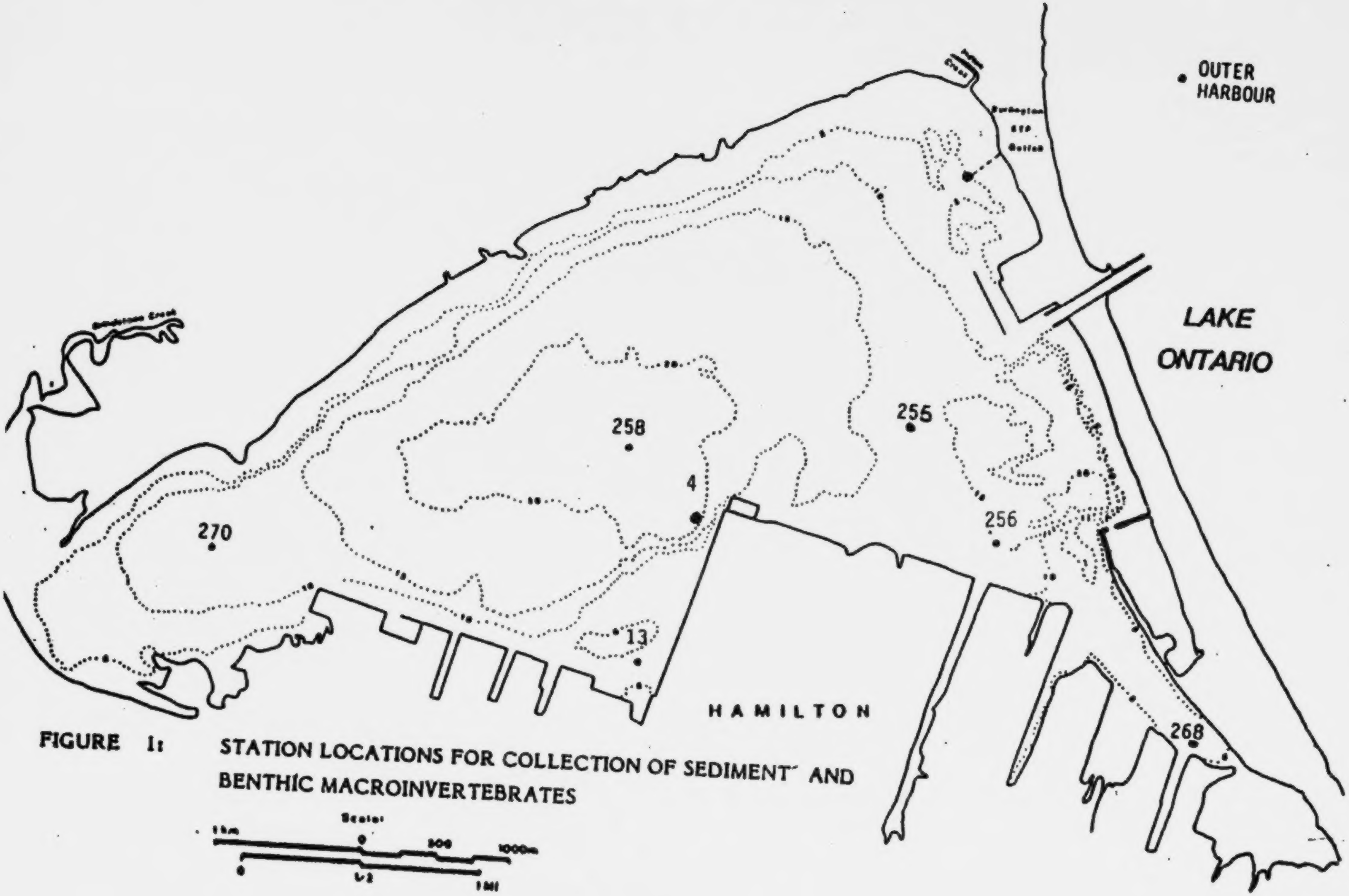

OUTER HARBOUR
Creek
Burlington
STP
Outfall
LAKE ONTARIO
258
255
4
270
256
13
268
HAMILTON
Scale:
1km
0
500
1000m
0
1/2
1MI

FIGURE 1: STATION LOCATIONS FOR COLLECTION OF SEDIMENT AND BENTHIC MACROINVERTEBRATES

Sediment pH, Eh and temperature were measured at time of collection. In order to minimize disturbance of the sediment, all measurements were performed while the sediment remained in the Ekman grab. The pH readings were taken with a Cole-Parment digital pH meter, while Eh was measured with an Orion millivolt meter equipped with a calomel electrode coupled with a salt bridge and a platinum electrode.

Collection of macroinvertebrates for analysis of community composition

During phase one, at each harbour station and for each visit, five Ekman grabs (22 cm x 22 cm) were collected, sieved through a 500 um screen and pooled in a 1 L container. Due to the nature of the sediment at the station in the outer harbour it was necessary to use a Ponar grab. All samples were preserved in 10% formalin and stained.

Samples were sorted under a stereomicroscope (10 x) into major taxonomic groups. Oligochaetes were identified to species while all other taxa were identified to genus with the exception of Nematoda, Turbellaria and Hydracarina. Chironomidae were decapitated and mounted in a permanent clearing mountant prior to identification. Oligochaetes were subsampled and 75 to 100 individuals from each sample were mounted in a permanent clearing mountant and identified to species. Species densities were expressed per meter squared and each species present in a sample was ranked according to its numerical dominance within that sample.

Toxicity evaluation experiment: chemical treatment of sediment

During phase two, at stations 270, 258, 256 and 13, sediment was treated with either iron, alum, oxygen, slag, or lime (Murphy, National Water Research Institute, pers. comm.). Untreated aliquots were also retained to permit a comparison of toxicity of the original sediment with that of treated material. Sediment treatements consisted of:

1. Oxygen bubbling to saturation
2. Slag addition of 5 $g.l^{-1}$ wet sediment
3. $FeCl_3$ addition of 250 $mg.l^{-1}$ wet sediment
4. Alum (Al_2SO_4) addition of 250 $mg.l^{-1}$ wet sediment
5. Lime ($CaOH_2$) addition of 250 $mg.l^{-1}$ wet sediment.

Sediment was treated for 6 weeks before beginning the bioassays. Jars were gently shaken once a week.

Sediment bioassays

Sediment bioassays employed a static beaker design. Test organisms were mayfly nymphs (Hexagenia limbata) weighing approximately 30 $mg.individual^{-1}$ (wet weight) and 3 to 4 month old juvenile fathead minnows (Pimephales promelas) weighing approximately 400 $mg.individual^{-1}$ wet weight. For the treated sediment experiments, egg-sac stage rainbow trout (Salmo gairdneri) were also used. Growth, mortality, and bioaccumulation of contaminants were the endpoints measured. The sediment bioassay protocol followed the method detailed by Krantzberg (1990a). Two-litre wide mouth glass jars of surface area 100 cm^2 were filled to a depth of 3 cm with sediment and 1,200 ml of deionized water to obtain a water:sediment ratio of 4:1 (v/v). The sediment and water mixtures were allowed to settle for 24 hours. Aeration was provided one hour prior to addition of the test organisms and continued throughout the duration of the experiment. Water loss due to evaporation was replaced as necessary to retain the appropriate volumetric ratio of water to sediment. Dissolved oxygen, pH, conductivity and temperature were monitored routinely during the experiments.

The exposure duration was 21 days at which time the beakers were harvested for surviving individuals. Ten individual mayflies or fathead minnows were allocated to triplicate bioassay chambers assembled for each station, each visit, and treated sediment. Triplicate containers of fifty egg-sac stage rainbow trout were prepared, and rainbow trout were suspended directly above the sediment in nylon mesh bags.

Initial biomass was estimated based on five randomly collected samples of organisms. Final biomass was determined for individual beakers. Where biomass was insufficient for chemical analysis, the biota for the triplicate beakers were pooled. Different species were not pooled together. Organisms for trace organic and metal analysis were wrapped in hexane-rinsed aluminum foil and plastic, respectively, and frozen until analysis. Honey Harbour sediment (Georgian Bay, Lake Huron), the site from which mayflies were collected for use in the bioassays, was used to monitor control growth and mortality.

Results and Discussion

Benthic community structure

All stations within Hamilton Harbour were dominated by low oxygen tolerant oligochaetes, primarily Limnodrilus hoffmeisteri, L. cervix, Tubifex tubifex and Quistradrilus multisetosus. Eh measurements of the sediment were all marginally positive, indicating that surface sediment was slightly oxic (Table 1), however Eh values could be misleading as a consequence of sediment handling, in spite of efforts to minimize disturbance of the sediment. The anoxic odour noted during collection suggests that the sediment would have had negative Eh values *in situ*.

Station 268 located at the mouth of Windermere Basin had the highest density of oligochaete species with 21,000 individuals.m^{-1}, indicative of high organic enrichment (Table 2). This station is in close proximity to the Woodward Avenue sewage treatment plant outfall. Macroinvertebrate communities at Station 255 and station 4 were dominated by immature tubificids without hair setae and are likely to be L. hoffmeisteri and L. cervix. Station 255 was the only station within the harbour where Gammarus was found. The presence of this organism is indicative of oxic conditions at the sediment-water interface.

The outer harbour station had the highest species diversity, with Pisidium being the dominant invertebrate. Species such as Potamothrix moldaviensis, P. vejdovskyi, Spirosperma ferox and Stylodrilus heringianus found in the outer harbour station are all oligochaete fauna that indicate mesotrophic conditions and moderately enriched sediment.

TABLE 1: PARAMETERS FOR HAMILTON HARBOUR BULK SEDIMENT SAMPLES

Station-Visit	Temperature (°C)	pH	Eh (MV)
270-1	6	7.11	+65
270-2	6	7.53	+85
258-1	7	7.36	+35
258-2	7	7.41	+45
255-1	7	7.28	+50
255-2	7	7.38	+45
4-1	6	7.40	+45
4-2	7	7.28	+60
268-1	9	6.61	+105
268-2	10	6.90	+125
Outer Harbour-1	4	7.58	+155
Outer Harbour-2	4	7.32	+105

TABLE 2. BENTHIC MACROINVERTEBRATE COMMUNITY COMPOSITION STATIONS SAMPLED NOVEMBER 1988

BENTHIC DATA FOR STATION 258, LOCATED IN THE CENTRAL REGION OF HAMILTON HARBOUR

Station 258

Visit	1		2	
Species	No./m^2	Rank	No./m^2	Rank
P. Coelenterata				
F. Hydridae				
Hydra sp.				
P. Platyhelminthes				
Cl. Turbellaria				
O. Tricladida sp. indet.				
P. Nematoda sp. indet.				
P. Annelida				
Cl. Oligochaeta				
F. Naididae				
Chaetogaster diaphanus				
Ophidonais serpentina				
Stylaria lacustris				
Arcteonais lomondi				
Dero nivea				
F. Tubificidae				
Tubifex tubifex	670	3	92	5
Potamothrix moldaviensis				
Potamothrix vejdovskyi				
Limnodrilus hoffmeisteri	2,197	2	4,015	1
Limnodrilus cervix				
Ilyodrilus templetoni				
Spirosperma ferox				
Quistadrilus multisetosus	287	5	375	4
Immature with hair setae	478	4	654	3
Immature without hair setae	3,819	1	1,776	2
F. Lumbriculidae				
Stylodrilus heringianus				
Cl. Hirudinea				
F. Glossiphoniidae				
Helobdella stagnalis				
P. Arthropoda				
Cl. Crustacea				
O. Amphipoda				
F. Gammaridae				
Gammarus sp.				
Cl. Arachnida				
O. Acarina sp. indet.				
Cl. Insecta				
O. Diptera				
F. Chironomidae				
pupae sp. indet.				
Procladius sp.				
Chironomus sp.				
Paratanytarsus sp.	4	6		
Micropsectra sp.				
Dicrotendipes sp.				
Heterotrissocladius sp.				
P. Mollusca				
Cl. Gastropoda				
F. Valvatidae				
Valvata piscinalis				
Valvata sincera sincera				
Cl. Pelecypoda				
F. Sphaeriidae				
Sphaerium sp.				
Pisidium sp.				
Total Number of Organisms	7,455		6,912	

BENTHIC DATA FOR STATION 4, LOCATED 500 m NORTHEAST OF RANDLE REEF IN HAMILTON HARBOUR

Station 4

Visit	1		2	
Species	**No./m²**	**Rank**	**No./m²**	**Rank**
P. Coelenterata				
F. Hydridae				
Hydra sp.				
P. Platyhelminthes				
Cl. Turbellaria				
O. Tricladida sp. indet.				
P. Nematoda sp. indet.			15	6
P. Annelida				
Cl. Oligochaeta				
F. Naididae				
Chaetogaster diaphanus				
Ophidonais serpentina				
Stylaria lacustris				
Arcteonais lomondi				
Dero nivea				
F. Tubificidae				
Tubifex tubifex	866	4	367	5
Potamothrix moldaviensis				
Potamothrix vejdovskyi				
Limnodrilus hoffmeisteri	1,603	3	367	4
Limnodrilus cervix	124	6		
Ilyodrilus templetoni				
Spirosperma ferox				
Quistadrilus multisetosus	493	5	612	3
Immature with hair setae	1,727	2	1,714	2
Immature without hair setae	4,564	1	5,985	1
F. Lumbriculidae				
Stylodrilus heringianus				
Cl. Hirudinea				
F. Glossiphoniidae				
Helobdella stagnalis				
P. Arthropoda				
Cl. Crustacea				
O. Amphipoda				
F. Gammaridae				
Gammarus sp.				
Cl. Arachnida				
O. Acarina sp. indet.				
Cl. Insecta				
O. Diptera				
F. Chironomidae				
pupae sp. indet.				
Procladius sp.				
Chironomus sp.				
Paratanytarsus sp.				
Micropsectra sp.				
Dicrotendipes sp.				
Heterotrissocladius sp.				
P. Mollusca				
Cl. Gastropoda				
F. Valvatidae				
Valvata piscinalis				
Valvata sincera sincera				
Cl. Pelecypoda				
F. Sphaeriidae				
Sphaerium sp.				
Pisidium sp.				
Total Number of Organisms	9,377		9,060	
Total Number of Taxa	6		6	

BENTHIC DATA FOR STATION 270, LOCATED AT THE WESTERN END OF HAMILTON HARBOUR

Station 270

Visit	1		2	
Species	No./m^2	Rank	No./m^2	Rank
P. Coelenterata				
F. Hydridae				
Hydra sp.				
P. Platyhelminthes				
Cl. Turbellaria				
O. Tricladida sp. indet.				
P. Nematoda sp. indet.				
P. Annelida				
Cl. Oligochaeta				
F. Naididae				
Chaetogaster diaphanus				
Ophidonais serpentina				
Stylaria lacustris				
Arcteonais lomondi				
Dero nivea				
F. Tubificidae				
Tubifex tubifex	69	6	46	6
Potamothrix moldaviensis				
Potamothrix vejdovskyi				
Limnodrilus hoffmeisteri	941	1	846	2
Limnodrilus cervix	543	3	1,026	1
Ilyodrilus templetoni	184	5	758	3
Spirosperma ferox				
Quistadrilus multisetosus	38	7	180	5
Immature with hair setae	291	4	46	7
Immature without hair setae	593	2	536	4
F. Lumbriculidae				
Stylodrilus heringianus				
Cl. Hirudinea				
F. Glossiphoniidae				
Helobdella stagnalis				
P. Arthropoda				
Cl. Crustacea				
O. Amphipoda				
F. Gammaridae				
Gammarus sp.				
Cl. Arachnida				
O. Acarina sp. indet.			4	9
Cl. Insecta				
O. Diptera				
F. Chironomidae				
pupae sp. indet.				
Procladius sp.				
Chironomus sp.				
Paratanytarsus sp.	8	8	4	8
Micropsectra sp.				
Dicrotendipes sp.				
Heterotrissocladius sp.				
P. Mollusca				
Cl. Gastropoda				
F. Valvatidae				
Valvata piscinalis				
Valvata sincera sincera				
Cl. Pelecypoda				
F. Sphaeriidae				
Sphaerium sp.				
Pisidium sp.				
Total Number of Organisms	2,617		3,446	
Total Number of Taxa	8		9	

BENTHIC DATA FOR STATION 255, LOCATED AT THE EASTERN END OF HAMILTON HARBOUR

Station 255

Visit	1		2	
Species	No./m^2	Rank	No./m^2	Rank
P. Coelenterata				
F. Hydridae				
Hydra sp.				
P. Platyhelminthes				
Cl. Turbellaria				
O. Tricladida sp. indet.				
P. Nematoda sp. indet.				
P. Annelida				
Cl. Oligochaeta				
F. Naididae				
Chaetogaster diaphanus				
Ophidonais serpentina				
Stylaria lacustris				
Arcteonais lomondi				
Dero nivea				
F. Tubificidae				
Tubifex tubifex	145	5		
Potamothrix moldaviensis				
Potamothrix vejdovskyi				
Limnodrilus hoffmeisteri	1,891	2	3,307	2
Limnodrilus cervix				
Ilyodrilus templetoni	145	6		
Spirosperma ferox				
Quistadrilus multisetosus	436	3	689	4
Immature with hair setae	436	4	1,714	3
Immature without hair setae	3,628	1	4,332	1
F. Lumbriculidae				
Stylodrilus heringianus				
Cl. Hirudinea				
F. Glossiphoniidae				
Helobdella stagnalis				
P. Arthropoda				
Cl. Crustacea				
O. Amphipoda				
F. Gammaridae				
Gammarus sp.	8	7	15	5
Cl. Arachnida				
O. Acarina sp. indet.				
Cl. Insecta				
O. Diptera				
F. Chironomidae				
pupae sp. indet.				
Procladius sp.				
Chironomus sp.				
Paratanytarsus sp.				
Micropsectra sp.				
Dicrotendipes sp.				
Heterotrissocladius sp.				
P. Mollusca				
Cl. Gastropoda				
F. Valvatidae				
Valvata piscinalis				
Valvata sincera sincera				
Cl. Pelecypoda				
F. Sphaeriidae				
Sphaerium sp.				
Pisidium sp.				
Total Number of Organisms	6,689		10,057	
Total Number of Taxa	7		5	

BENTHIC DATA FOR STATION 268, LOCATED AT THE MOUTH OF WINDERMERE BASIN IN HAMILTON HARBOUR

Station 268

Visit	1		2	
Species	No./m^2	Rank	No./m^2	Rank
P. Coelenterata				
F. Hydridae				
Hydra sp.				
P. Platyhelminthes				
Cl. Turbellaria				
O. Tricladida sp. indet.				
P. Nematoda sp. indet.	153	11	31	11
P. Annelida				
Cl. Oligochaeta				
F. Naididae				
Chaetogaster diaphanus	31	12	31	12
Ophidonais serpentina			107	9
Stylaria lacustris	15	13		
Arcteonais lomondi				
Dero nivea	1,209	6	321	6
F. Tubificidae				
Tubifex tubifex	5,771	2	3,169	1
Potamothrix moldaviensis				
Potamothrix vejdovskyi				
Limnodrilus hoffmeisteri	7,210	1	2,541	2
Limnodrilus cervix	3,613	3	1,378	3
Ilyodrilus templetoni	475	7		
Spirosperma ferox				
Quistadrilus multisetosus	1,209	5	214	7
Immature with hair setae	245	10	429	5
Immature without hair setae	1,929	4	1,072	4
F. Lumbriculidae				
Stylodrilus heringianus				
Cl. Hirudinea				
F. Glossiphoniidae				
Helobdella stagnalis				
P. Arthropoda				
Cl. Crustacea				
O. Amphipoda				
F. Gammaridae				
Gammarus sp.				
Cl. Arachnida				
O. Acarina sp. indet.				
Cl. Insecta				
O. Diptera				
F. Chironomidae				
pupae sp. indet.	15	14		
Procladius sp.	383	8	61	10
Chironomus sp.	352	9	122	8
Paratanytarsus sp.				
Micropsectra sp.				
Dicrotendipes sp.				
Heterotrissocladius sp.				
P. Mollusca				
Cl. Gastropoda				
F. Valvatidae				
Valvata piscinalis	3	15		
Valvata sincera sincera				
Cl. Pelecypoda				
F. Sphaeriidae				
Sphaerium sp.				
Pisidium sp.				
Total Number of Organisms	22,613		9,476	
Total Number of Taxa	15		12	

BENTHIC DATA FOR THE CONTROL STATION, LOCATED 1 km NORTHEAST FROM THE MOUTH OF HAMILTON HARBOUR

Station: Control

Visit	1		2	
Species	No./m^2	Rank	No./m^2	Rank
P. Coelenterata				
F. Hydridae				
Hydra sp.	122	13	15	17
P. Platyhelminthes				
Cl. Turbellaria				
O. Tricladida sp. indet.	46	16	46	11
P. Nematoda sp. indet.	15	19		
P. Annelida				
Cl. Oligochaeta				
F. Naididae				
Chaetogaster diaphanus				
Ophidonais serpentina	138	12	31	16
Stylaria lacustris				
Arcteonais lomondi	260	9		
Dero nivea				
F. Tubificidae				
Tubifex tubifex				
Potamothrix moldaviensis	3,199	3	107	6
Potamothrix vejdovskyi	3,337	2	459	5
Limnodrilus hoffmeisteri				
Limnodrilus cervix				
Ilyodrilus templetoni				
Spirosperma ferox	398	7	31	15
Quistadrilus multisetosus	536	6	31	14
Immature with hair setae			31	13
Immature without hair setae	2,526	5	490	4
F. Lumbriculidae				
Stylodrilus heringianus	3,062	4	1,255	2
Cl. Hirudinea				
F. Glossiphoniidae				
Helobdella stagnalis	15	21		
P. Arthropoda				
Cl. Crustacea				
O. Amphipoda				
F. Gammaridae				
Gammarus sp.			15	18
Cl. Arachnida				
O. Acarina sp. indet.	46	15	46	10
Cl. Insecta				
O. Diptera				
F. Chironomidae				
pupae sp. indet.			15	19
Procladius sp.	153	10	107	7
Chironomus sp.	15	20	61	8
Paratanytarsus sp.				
Micropsectra sp.	153	11	46	12
Dicrotendipes sp.	31	18		
Heterotrissocladius sp.	31	17		
P. Mollusca				
Cl. Gastropoda				
F. Valvatidae				
Valvata piscinalis				
Valvata sincera sincera	[illegible]	8	382	3
Cl. Pelecypoda				
F. Sphaeriidae				
Sphaerium sp.	100	14	61	9
Pisidium sp.	4,087	1	2,832	1
Total Number of Organisms	18,668		6,261	
Total Number of Taxa	21		19	

Sediment bioassays on untreated sediment

Fathead Minnows:

Hamilton Harbour sediment was not lethal to fathead minnows with the exception of station 268 where complete mortality occurred within 2 days of exposure. This rapid lethality was likely due to elevated ammonia concentrations associated with the Woodward Avenue sewage treatment plant effluent. Aqueous chemical measurements generated by the Ontario Ministry of the Environment in the fall of 1988 close to the date when sediment samples were collected showed ammonium concentrations in excess of 6 $mg.l^{-1}$. This would result in at least 0.07 $mg.l^{-1}$ of unionized ammonia (at pH 7.5, 20 C) which exceeds the provincial guideline of 0.02 $mg.l^{-1}$. Subsequent bioassays were performed using sediment elutriates to differentiate between toxicity associated with solid and aqueous phases. Elutriates were prepared by continuous shaking of four parts dechlorinated, deionized tap water with one part sediment for one hour. The supernatant rapidly elicited mortality, supporting the theory that ammonia, and not contaminants more typically associated with the solid phase, was responsible for the lethal nature of the substrate.

Biomass decreased during the duration of the exposure, and was variable between sediment collected at the two visits (Table 3). This could be due to subtle differences in the nutrient composition of sediment collected at each visit. Sediment in Hamilton Harbour was higher in organic content than the control sediment (Table 4) and biomass loss in controls was generally comparable to test sediment. In some cases (Station 4, Station 255) fathead minnows lost less

Table 3. SEDIMENT BIOASSAY RESULTS ON UNTREATED HAMILTON HARBOUR SEDIMENT, 1988.

	Hexagenia limbata		Pimephales promelas	
STATION	% MORTALITY (s.d.)	BIOMASS CHANGE (mg)	% MORTALITY (s.d.)	BIOMASS CHANGE (mg)
OH-1	11 (11)	21.3	0 (0)	-27.3
OH-2	30 (26)	-3.7	0 (0)	-64.0
4-1	19 (23)	17.0	7 (11)	-24.7
4-2	22 (11)	24.3	7 (11)	2.0
255-1	7 (6)	6.0	13 (11)	-9.0
255-2	22 (19)	6.3	7 (11)	-26.7
258-1	19 (17)	22.7	0 (0)	-42.7
258-2	0 (0)	19.7	0 (0)	-28.7
268-1	11 (11)	14.3	100 (0)	–
268-2	15 (13)	20.3	100 (0)	–
270-1	4 (6)	20.3	0 (0)	-72.0
270-2	4 (6)	15.7	0 (0)	-29.3
CONTROL	7 (12)	13.0	0 (0)	-52.0

TABLE 4 HAMILTON HARBOUR SEDIMENT CHEMISTRY, 1988 - 1989

SUBSTANCE	270	255	258	4	13	268	256	HONEY HARBOUR	CONTROL (OUTER HARBOUR)
Zn	2.05E+03	4.06E+03	4.61E+03	2.19E+03	1.66E+03	2.03E+03	3.27E+03	1.55E+02	1.07E+02
Cd	9.26E+00	1.52E+01	1.53E+01					4.61E+00	4.64E+00
Pb	2.86E+02	4.69E+02	5.08E+02	2.70E+02	1.50E+02	3.50E+02	4.90E+02	7.65E+01	7.51E+01
Ni	5.31E+01	7.30E+01	7.31E+01	4.70E+01	3.40E+01	4.90E+01	3.70E+01	3.49E+01	2.60E+01
Fe	6.41E+04	1.49E+05	1.11E+05					4.50E+04	3.92E+04
Mn	1.77E+03	2.78E+03	2.71E+03					1.05E+03	8.52E+02
Cr	1.40E+02	3.26E+02	2.64E+02	2.00E+02	1.25E+02	4.00E+02	4.90E+02	5.10E+01	4.45E+01
Cu	1.10E+02	2.06E+02	1.75E+02	9.50E+01	9.50E+01	1.60E+02	8.50E+01	2.40E+01	2.57E+01
Al	4.06E+04	2.61E+04	3.21E+04					3.06E+04	3.81E+04
As	1.12E+01	1.20E+01	1.46E+01	2.40E+01	1.00E+01	5.00E+00	3.30E+01	1.50E+00	
PCB	3.65E-01	2.31E-01	6.22E-01	1.54E-01	4.67E-01	8.60E-01	4.58E-01		
NAPTHALENE	6.50E-01	3.30E-01	3.58E+00	3.04E+00	4.91E+00	2.26E+00	7.47E+00		
PHENANTHRENE	1.17E+00	1.32E+00	4.12E+00	5.54E+01	1.49E+01	2.57E+00	1.55E+01		
PYRENE	2.05E+00	1.56E+00	6.33E+00	6.53E+01	2.78E+01	7.59E+00	3.45E+01		
FLOURENE	2.50E-01	2.45E+00	1.54E+00	2.87E+00	1.07E+00	5.90E-01	2.90E-01		
FLOURANTHENE	2.54E+00	1.40E+01	1.26E+01	3.41E+01	9.39E+00	5.47E+00	1.85E+00		
%TOC	4.36E+00	6.37E+00	6.27E+00	9.39E+00	9.27E+00	8.55E+00	8.10E-01	2.68E+00	3.55E+00

weight in Hamilton Harbour sediment.

Thus, despite the high concentrations of trace metals, sediment was virtually non toxic to fathead minnows. Complexation of metals with either iron or sulfur compounds may be restricting the bioavailability of metals to these organisms (Jenne 1968, Di Toro et al 1990, Tessier et al 1984). Extrordinarily high Fe concentrations reflect the prsence of metal smelting at the harbour.

Mayflies:

Mayfly mortality greater than controls was observed at all stations with the exception of station 270, despite the high concentrations of Zn, Cd and Pb at this location. The outer harbour station elicited the greatest mortality when sediment from the second visit was assessed. This was attributed to the extremely sandy nature of the substrate which rendered it unsuitable for mayfly burrowing. Mayfly mortality varied among replicates and between station visits. The mean coefficient of variation between visits for mortality was 12 % ± 7%, however, the only significant difference in mortality between visits was observed at station 258. The degree of sediment toxicity observed at these stations is in agreement with the toxicity zone map presented by Rodgers et al. (1989).

With the exception of OH-2, all mayflies increased in biomass. Growth was apparently depressed in Station 255 sediment relative to controls. This was the only station where fathead minnow mortality exceeded 10%. Tissue residues of As, Cd, Cr, Cu, Pb and Zn tended to be the highest in mayflies exposed to Station 255 sediment as compared with those exposed to sediment from other stations. Metal toxicity could be the source of the impaired growth.

In general, tissue residues of Cd, Cr, Cu, Pb, Ni and Zn were higher in organisms exposed to Hamilton Harbour sediment than in organisms from control sediment (Table 5). PAHs were also accumulated by test organisms, however, other trace organic contaminants were not detectable, marginally above the detection limit, or not significantly different from controls (Tables 6, 7). While the lack of significant accumulation of PCBs and other compounds in encouraging, it is possible that the 21 day exposure interval was not sufficient to reveal the accumulation of higher molecular weight compounds. Kannan et al (1989) found that time to 90% uptake equilibrium of some highly clorinated coplaner PCB ranged from 31 to 85 days for the mussel Perna viridis Linnaeus. Similarly, steady state for PCB 1254 was not reached until day 29 by the prawn Macrobranchium renbergii and the clam Corbicula fluminea (Tatem 1986). Longer exposure to Hamilton Harbour sediment, then, may have resulted in higher PCB concentrations in test organisms.

Treated sediment, toxicity evaluation experiment

Hexagenia limbata:

The effectiveness of the chemical treatments varied among sediment samples (Figure 2). For the two highly toxic sediment samples, stations 13 and 256, lethality was ameliorated by chemical treatment. The lethality of station 13 sediment was moderately decreased by dosing with lime. This station had the highest concentration of PAHs in sediment and this conformed with the highest PAH concentrations measured in tissues of mayflies. PAHs may have been important in contributing to the toxicity of this sediment, and treatments were only

TABLE 5. METALS IN FATHEAD MINNOWS AND MAYFLY NYMPHS FROM UNTREATED HAMILTON HARBOUR SEDIMENT, 1988/1989

FATHEAD MINNOWS

STATION	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Hg	Ni	Zn
255-1	884	1.47	0.363	11.6	26.1	2889	15.1	109	0.41	4.8	381
255-2	329	1.18	0.147	5.0	21.5	1224	5.5	48	0.48	3.1	273
258-1	579	1.95	0.379	8.2	21.8	2158	13.7	112	0.37	4.1	368
258-2	481	1.19	0.238	6.3	20.3	1794	10.5	103	0.41	2.7	268
270-1	970	1.67	0.452	8.9	25.0	2494	13.1	134	0.37	7.4	329
270-2	1433	2.06	0.928	12.6	27.1	3978	20.8	224	0.37	7.1	427
4-1	390	1.10	0.190	5.4	19.4	1429	8.5	97	0.35	2.2	278
4-2	6776	1.47	0.329	9.8	24.2	2912	13.3	164	0.41	0.7	349
13-1			0.422	6.5	6.8		5.3				183
13-2			0.511	7.2	10.3		4.4				212
CONTROL	355	0.80	0.055	1.2	14.2	660	1.0	23	0.47	1.6	202
CULTURE	7	0.52	0.021	0.4	6.4	63	0.3	2	0.18	0.9	84
OH1	994	1.06	0.206	4.9	22.3	1806	4.2	51	0.38	4.9	221
OH2	868	1.05	0.111	2.5	17.8	1516	2.3	59	0.38	1.9	235

TABLE 5. METALS IN FATHEAD MINNOWS AND MAYFLY NYMPHS FROM UNTREATED HAMILTON HARBOUR SEDIMENT, 1988/1989

MAYFLIES

STATION	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Hg	Ni	Zn
255-1	2253	13.25	1.699	35.1	41.0	11458	58.7	367	0.22	1.5	664
255-1	1888	6.13	1.463	29.5	38.4	10213	49.8	328	0.15	13.9	609
255-2	1600	4.60	1.350	28.3	35.6	8710	44.3	296	0.11	11.3	584
258-1	1770	5.60	1.280	24.7	29.9	8380	43.2	379	0.11	12.1	591
258-2	1982	7.73	1.245	26.2	32.9	10518	49.5	426	0.15	14.2	552
268-1	1604	2.19	0.927	30.0	38.9	5250	24.1	119	0.19	8.8	297
268-2	1323	1.01	1.393	20.1	34.2	4162	16.7	107	0.10	4.8	280
270-1	1969	5.26	1.031	16.7	30.7	5835	36.3	300	0.12	12.1	451
270-1	2134	5.67	1.062	18.4	31.5	6920	37.6	307	0.19	1.2	472
270-2	3104	6.04	1.521	26.6	36.1	9031	57.8	449	0.14	15.3	679
4-1	1967	3.04	1.326	22.1	31.0	8837	42.4	422	0.19	12.1	538
4-2	1764	5.79	1.100	18.9	27.5	8045	42.4	384	0.12	11.7	417
13-1			2.950	29.1	18.9		42.2				624
13-2			2.370	24.9	18.7		74.6				752
CONTROL	3689	2.56	0.611	8.0	18.0	6700	9.2	282	0.08	8.6	190
CULTURE	690	0.80	0.850	2.4	15.7	1416	2.2	98	0.07	2.9	202
OH1	2884	3.26	0.979	11.4	28.5	5474	21.7	203	0.09	10.1	208
OH2	2521	3.54	1.354	7.2	27.5	4479	11.9	236	0.07	9.0	242

all values in ug/g dry weight

'ABLE 6. PAHs IN MAYFLIES AND FATHEAD MINNOWS IN UNTREATED HAMILTON HARBOUR SEDIMENT 1988/1989. ALL VALUES IN UG/G WET WEIGH

FATHEAD MINNOWS

STATION	PHENANTHRENE	FLOURANTHENE	PYRENE	BENZO(a)-ANTHRACENE	BENZO(e) PYRENE	BENZO(b) FLOURANTHENE	BENZO(k)-FLOURANTHENE	BENZO(a) PYRENE	BENZO(ghi)-PERYLENE	DIBENZ(ah)-ANTHRACENE	IND(123-cd)-PYRENE
255-1	0.313	0.084	0.128	0.017	0.000	0.017	0.000	0.006	0.000	0.000	0.000
255-2	0.594	0.185	0.238	0.000	0.043	0.040	0.000	0.019	0.000	0.000	0.000
258-1	0.312	0.073	0.109	0.279	0.000	0.000	0.000	0.011	0.000	0.000	0.000
258-2	0.423	0.049	0.094	0.155	0.000	0.000	0.000	0.011	0.000	0.000	0.000
268-1	0.836	0.847	0.577	0.147	0.008	0.175	0.032	0.209	0.028	0.000	0.029
270-1	0.122	0.020	0.034	0.097	0.000	0.000	0.000	0.008	0.000	0.000	0.000
270-2	0.092	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4-1	1.412	0.288	0.401	0.063	0.055	0.058	0.000	0.055	0.051	0.000	0.012
4-2	0.234	0.043	0.073	0.006	0.000	0.007	0.000	0.020	0.000	0.000	0.000
19-1	0.000	0.150	0.271	0.400	0.052	0.373	0.000	0.054	0.062	0.032	0.121
OH-1	0.073	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
OH-2	0.056	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CONTROL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CULTURE	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

ABLE 6. PAHs IN MAYFLIES AND FATHEAD MINNOWS IN UNTREATED HAMILTON HARBOUR SEDIMENT 1988/1989. ALL VALUES IN UG/G WET WEIGH

MAYFLIES

STATION	PHENANTHRENE	FLOURANTHENE	PYRENE	BENZO(a)-ANTHRACENE	BENZO(e) PYRENE	BENZO(b) FLOURANTHENE	BENZO(k)-FLOURANTHENE	BENZO(a) PYRENE	BENZO(ghi)-PERYLENE	DIBENZ(ah)-ANTHRACENE	IND(123-cd)-PYRENE
255-1	1.150	0.826	0.840	0.273	0.014	0.227	0.092	0.342	0.057	0.000	0.040
255-2	1.827	1.361	1.368	0.473	0.030	0.390	0.122	0.585	0.094	0.006	0.071
258-1	0.041	0.022	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
258-2	1.000	0.708	0.725	0.000	0.013	0.258	0.037	0.340	0.058	0.000	0.040
268-1	0.475	0.569	0.409	0.267	0.000	0.117	0.013	0.135	0.020	0.000	0.017
270-1	0.376	0.271	0.259	0.000	0.006	0.152	0.005	0.174	0.022	0.000	0.010
270-2	0.283	0.191	0.182	0.000	0.000	0.106	0.000	0.117	0.019	0.000	0.011
4-1	3.115	1.946	1.984	0.489	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4-2	2.634	1.373	4.412	0.455	0.260	0.425	0.149	0.536	0.080	0.008	0.074
13-1	0.120	1.172	1.069	2.227	1.571	1.259	0.880	1.572	0.302	0.201	0.458
13-2	0.290	8.460	5.660	4.310	1.595	1.374	0.938	1.555	0.350	0.103	0.333
OH-1	0.084	0.048	0.033	0.012	0.000	0.013	0.000	0.004	0.000	0.000	0.000
OH-2	1.743	1.413	1.399	0.482	0.027	0.417	0.161	0.625	0.107	0.014	0.078
CONTROL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CULTURE	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

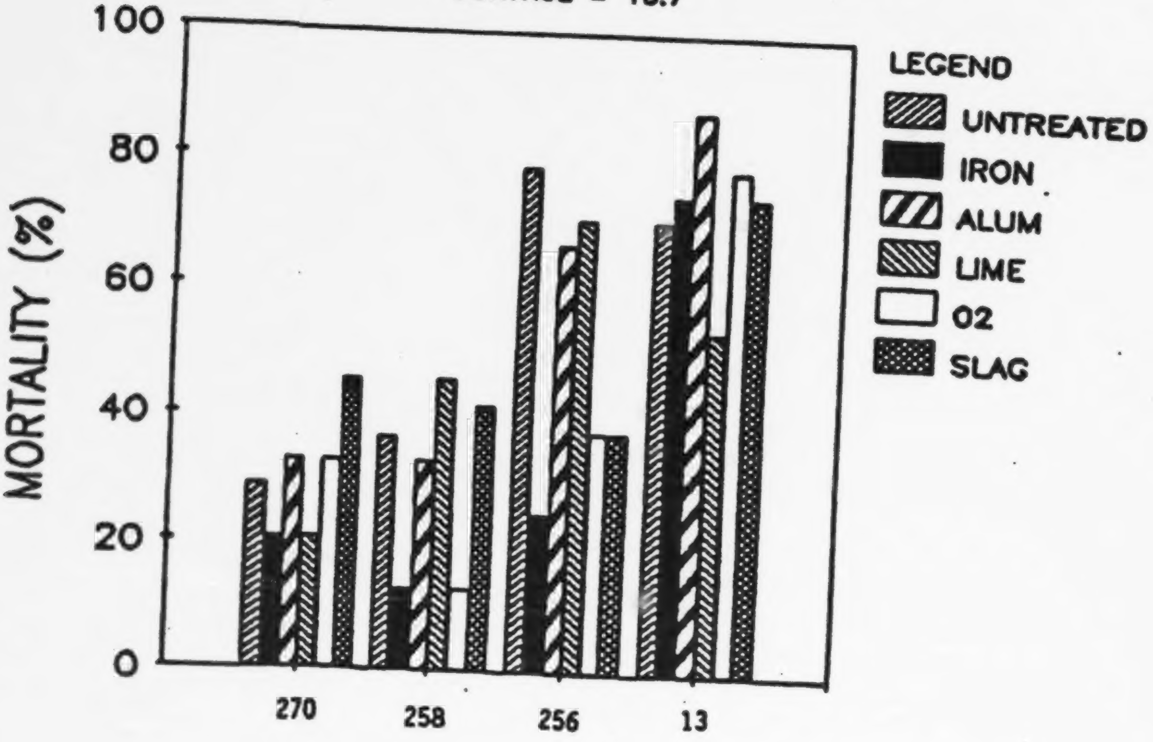
Table 7 Trace organic compounds measured in fathead minnows and mayflies exposed to Hamilton Harbour sediment (ND = not detectable, T = trace: < 0.03 ug.g^{-1}, C = comparable to controls, D = detected)

COMPOUND	STATUS	DETECTION LIMIT
pp-DDE	T	0.003
pp-DDD	ND	0.010
pp-DDT	ND	0.010
op-DDT	T	0.010
a-ENDOSULFAN	ND	0.003
b-ENDOSULFAN	ND	0.005
DIELDRIN	T	0.003
ENDRIN	ND	0.003
HEXACHLOROETHANE	D	0.001
135-TRICHLOROBENZENE	T	0.002
124-TRICHLOROBENZENE	T	0.002
HEXACHLOROBUTADIENE	T	0.001
123-TRICHLOROBENZENE	T	0.002
1235-TRICHLOROBENZENE	ND	0.002
1245-TRICHLOROBENZENE	ND	0.002
26a-TRICHLOROTOLUENE	T	0.005
1234-TRICHLOROBENZENE	ND	0.001
PENTACHLOROBENZENE	ND	0.001
HEXACHLOROBENZENE	ND	0.001
HEPTACHLOR	T	0.005
HEPTACHLOREPOXIDE	ND	0.003
ALDRIN	C,T	0.005
MIREX	ND	0.005
a-BHC	C,T	0.005
b-BHC	C,T	0.010
d-BHC	ND	0.005
a-CHLORDANE	T	0.003
g-CHLORDANE	T	0.003
TOXAPHENE	ND	0.500
PCB	D,C	0.050
PHENANTHRENE	D	0.005
FLOURANTHENE	D	0.005
PYRENE	D	0.005
BENZO(a)ANTHRACENE	D	0.005
BENZO(e)PYRENE	D	0.005
BENZO(b)FLOURANTHENE	D	0.005
BENZP(k)FLOURANTHENE	D	0.005
BENZO(a)PYRENE	D	0.005
BENZO(ghi)PERYLENE	D	0.005
DIBENZ(ah)ANTHRACENE	T	0.005
IND(123-cd)PYRENE	D	0.005

FIGURE 2

HEXAGENIA BIOASSAY (21 DAYS) PERCENT MORTALITY IN TREATED AND UNTREATED SEDIMENTS

CONTROL = 16.7



marginally effective at reducing mortality. The lethality of station 256 sediment was decreased by oxygen, slag and iron treatments. Oxygen and slag also decreased the lethality of sediment from station 258, while no treatments mitigated lethality of sediment from station 270. The effectiveness of the treatments at stations 256 and 258 may be due to the enhanced chelation of trace metals and consequent reduction of bioavailability. The lack of any marked effects due to treatment of station 270 sediment may pertain to its demonstrated low level of initial toxicity. That is, if a substantial portion of the metals was not in a bioavailable form, treatment would not further reduce bioavailability, and therefore, toxicity.

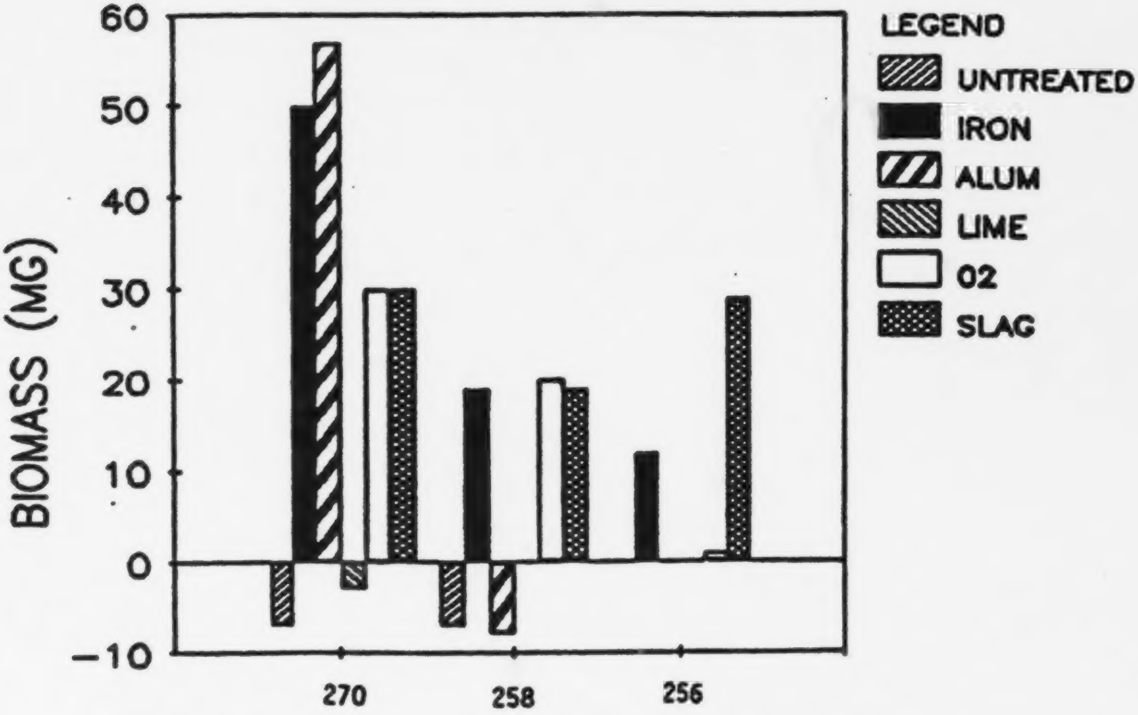
Growth was calculated when mortality did not exceed 50%. When mortality exceeded 50%, the biomass changes for the survivors were not brought into consideration. The high lethality of the sediment was considered to be of primary environmental significance, making calculations of growth inhibition superfluous. In addition, measurements of growth on the surviving individuals would have been highly variable due to the small sample size. While efforts were made to use organisms of standard biomass, some variability was inevitable. Differential survival of organisms that began the experiment slightly larger than the mean biomass would bias the results.

Growth of Hexagenia was suppressed in all untreated sediment relative to the control (Honey Harbour) sediment. Treatment of sediment from station 258 by oxygen, slag, and iron improved growth relative to that observed in the untreated sediment (Figure 3). Beneficial effects of oxygen, slag and iron, as well as alum, on growth of mayflies relative to the untreated sediment were observed for

FIGURE 3

HEXAGENIA BIOASSAY (21 DAYS) BIOMASS CHANGE IN TREATED AND UNTREATED SEDIMENTS

CONTROL = 2 MG (%M > 50 AT 13)



sediment from station 270. The success of the chemical treatments in alleviating growth inhibition may again be attributed to the reduction of metal bioavailability in sediment, and in the case of the oxygen treatment, to the direct improvement in substrate suitability for colonization by reducing sediment oxygen demand.

Salmo giardneri:

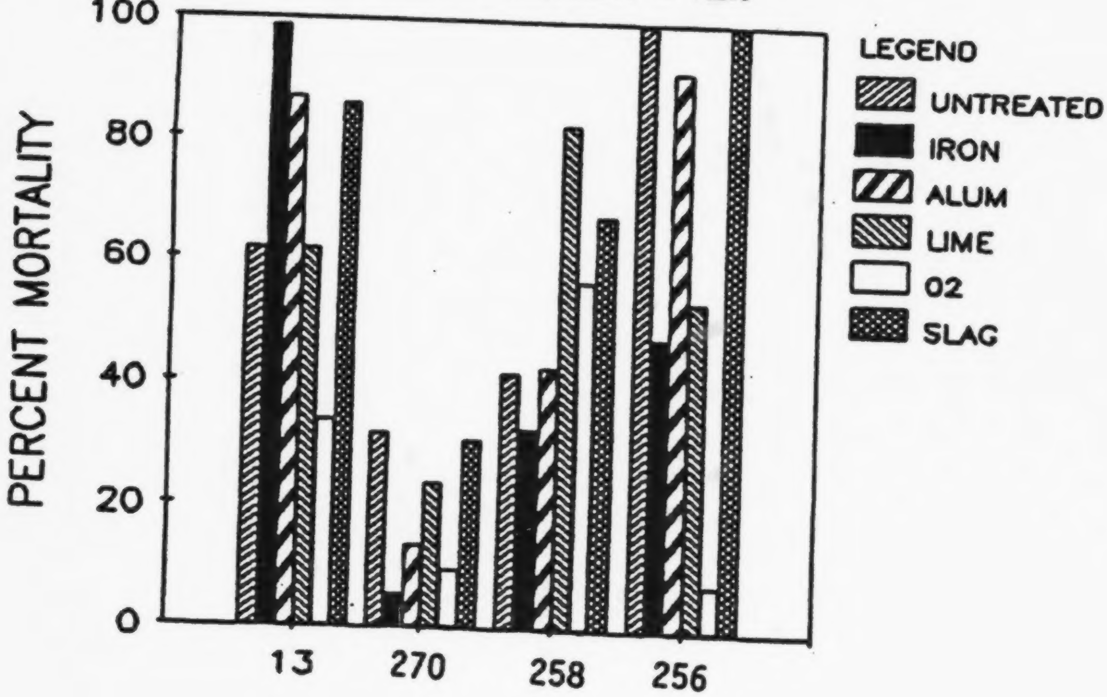
Egg-sac stage rainbow trout were particularly sensitive to depressed oxygen concentrations. Mortality was frequently associated with oxygen depression in overlying water below 5 $mg.L^{-1}$. As a consequence, variability between replicates due to insufficient dissolved oxygen concentrations confounded the assessment of treatment effects on mortality. In addition, no effects on growth were assessed in view of the limited number of fish surviving at the end of the bioassay.

As observed in the bioassays conducted with Hexagenia, mitigation of adverse consequences of exposure to the highly toxic sediment from stations 13 and 256 was provided by some of the treatments (Figure 4). However, not all treatments were equally beneficial to both species. Differential effectiveness may reflect alternate routes of exposure to contaminants, with mayflies ingesting sediment as compared to passive accumulation by the egg sac stage rainbow trout. The disparate responses also point out the value of examining the response of several organisms to test sediment. For example, treatment of sediment 13 with lime was beneficial for mayfly survival but not for rainbow trout survival. Instead, oxygen was found to ameliorate toxicity of sediment 13 to S. giardneri. This may have been a direct consequence of alleviating the high sediment oxygen

FIGURE 4

RAINBOW TROUT BIOASSAY
PERCENT MORTALITY IN TREATED
AND UNTREATED SEDIMENTS

CONTROL MORTALITY = 7.3%



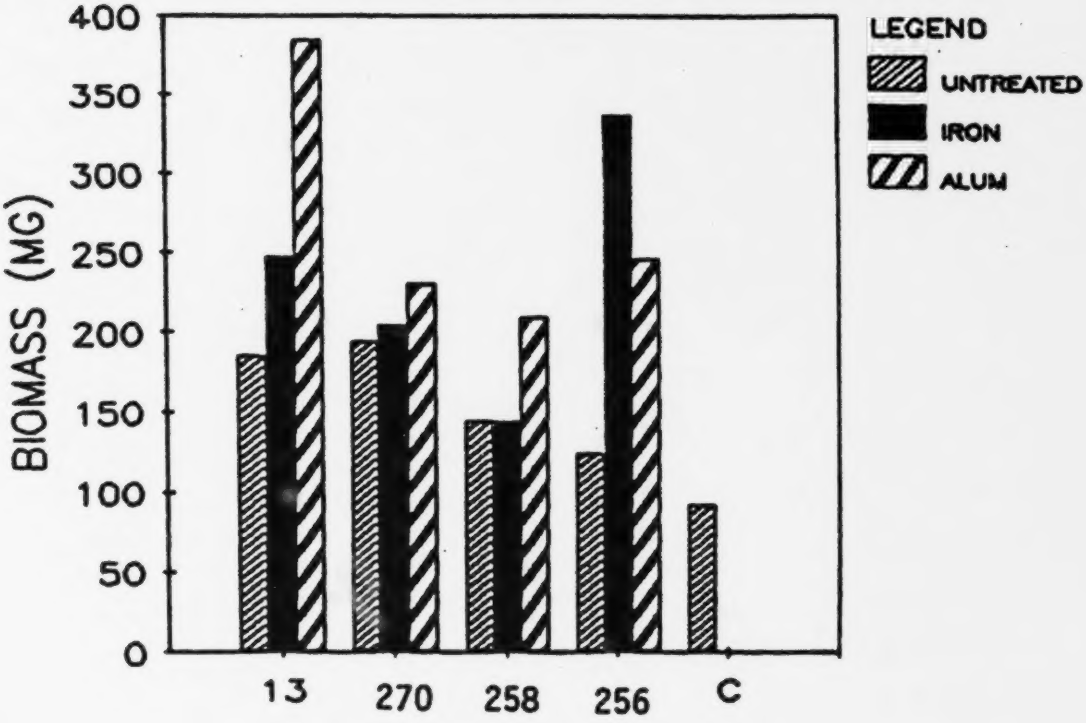
demand, rather than immobilizing contaminants. While oxygen, iron and slag lessened the toxicity of sediment 256 to mayflies, oxygen, iron and lime were effective in reducing mortality of rainbow trout. Oxygen, iron and alum decreased the toxicity of sediment from stations 258 and 270 relative to untreated sediment.

Pimephales promelas:

Fathead minnows were tested using untreated sediment and sediment treated with iron and alum. No mortality was observed in fathead minnow bioassays. In all cases, fathead minnows lost weight during the experiments. Minnows exposed to the untreated sediment, however, experienced a greater net loss in weight relative to the controls (Figure 5). The treatments were not effective at reducing sediment toxicity. Since minnows graze on sediment during the course of the experiment, the treatments may have rendered sediment less palatable, or bound nutrients as well as contaminants, resulting in no net improvement in toxicity.

The lethal and sublethal toxicity of Hamilton Harbour sediment to mayflies mirrored the restricted benthic fauna. Accumulation of metals and PAHs in test organisms in excess of controls suggests that contaminants would continue to limit benthic invertebrates *in situ*, even if hypolimnetic oxygen was completely restored. There was some evidence from the rainbow trout assay that high sediment oxygen demand could be restricting the biota, either directly, or indirectly by altering contaminant availability (Krantzberg 1990b). Portt et al (1989) were unable to correlate the abundance and composition of the benthic community with contaminant concentrations because of auto-correlations between

FIGURE 5
FATHEAD MINNOW BIOASSAY
BIOMASS LOSS IN TREATED
AND UNTREATED SEDIMENTS



contaminant concentrations, organic composition and grain size of the sediment, depth and dissolved oxygen.

Conclusions

The chemical treatments were effective in abating sediment toxicity in some instances. The substances used are known to chelate metals, and this assists in identifying the source or cause of the toxicity. Clearly, the techniques for dosing sediment with the intention of reducing toxicity, or identifying the cause of the observed toxicity, require further development before standard toxicity reduction experiments (TRE) on whole sediment become routine.

This investigation generated several noteworthy directions for future research. It is clear that different organisms respond in different manners, perhaps as a consequence of their different life histories. Knowledge of an organism's life history can provide clues as to the mode of uptake of contaminants. Sediment in areas of the harbour that have high concentrations of metals are not necessarily toxic, and this may reflect low bioavailability of contaminants. More sensitive measurements of the bioavailable portion of metals in sediment are needed.

While the macroinvertebrate community is clearly restricted as a consequence of summer anoxia, metal contamination in some areas of the harbour is a substantive issue. This is supported by the elevated tissue residues in those bioassay organisms that experienced adverse effects of Hamilton Harbour sediment. Similarly, PAH contamination is of concern given the pronounced tissue residues and toxicological responses of test organisms exposed to contaminated sediment from some locations.

Acknowledgements

Analysis of treated sediment was made possible by the cooperation of T. Murphy of the National Water Research Institute. S. Petro of Environment Ontario and B.A.R. Environmental Consultants conducted the sediment bioassays, and BEAK Consultants Ltd. collected and identified the benthic invertebrates. Members of the Water Resources Branch provided valuable suggestions in their review of the manuscript.

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EVALUATION OF THE APPARENT EFFECTS THRESHOLD (AET) AS A BASIS FOR SETTING MARINE SEDIMENT QUALITY CRITERIA.

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The AET approach utilizes measured concentrations of contaminants in marine sediments and parallel sediment toxicity data to predict adverse biological effects. There is a strong impetus in the U.S., and in Canada, to use the AET values as a basis for setting marine sediment quality criteria.

A recently completed research program for the American Petroleum Institute was designed to evaluate several aspects of the AET approach. Bioavailability and toxicity for a range of pollutants were evaluated in laboratory amphipod bioassays with a range of doses of contaminated sediments from Puget Sound. Despite the exceedance of the AETs for several metals and the observed bioaccumulation of certain PAHs, we observed no significant amphipod toxicity with the test sediments. These data suggest that sediment toxicity cannot be readily attributed to individual contaminants in complex mixtures.

A MULTIVARIATE APPROACH FOR DEFINING SPATIAL IMPACTS USING THE SEDIMENT QUALITY TRIAD.

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A concurrent assessment of benthic infaunal community structure, sediment chemistry, and sediment toxicity (known as the Sediment Quality Triad), was employed to develop an objective, multivariate approach to evaluate the spatial extent of benthic impact in an aquatic environment. Data acquired from 20 stations in Burrard Inlet, B.C. included 221 invertebrate taxa abundance estimates, 30 sediment chemical measures, and 7 toxicological responses. A Principal Components Analysis was performed on the two response attributes (community structure and sediment toxicity) to reduce their multidimensionality, then relate the observed spatial response patterns to the measured sediment levels for each chemical. Significant correlations ($p<0.001$) for cadmium, copper, hydrocarbons, and sediment volatile residue were documented for spatial changes in benthic community structure. Few positive toxic responses were observed. Using simple linear regressions, the predicted benthic response for selected sediment quality criteria for cadmium and copper, were obtained. Predicted spatial impacts (i.e. criteria exceeded) were portrayed graphically. The feasibility of this approach for use in data management and for regulatory purposes was discussed.

LONG-TERM EXPOSURE OF *Neanthes* TO TOXIC MARINE SEDIMENT

ABSTRACT

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The effect of long-term exposure of juvenile *Neanthes arenaceodentata* to toxic marine sediments was examined to determine the relationship between changes in juvenile biomass and endpoints associated with reproductive success. At the end of a 25 day exposure period, the highest average individual biomass reported was for the West Beach (WB) control and Carr Inlet (CI) reference sediments, with the lowest biomass reported for Elliot Bay (EB). As the amount of contamination (determined by the EB fraction in the test sediment) increased, biomass decreased to almost zero growth in the most contaminated sediment (100 percent). Delay in reaching sexual maturity increased as the contamination increased. Worms exposed to WB and CI sediments matured between days 20 and 25, whereas worms exposed to the EB or an equal volume mixture of CI and EB sediments (CI/EB) matured between days 25 and 32. EB male and female worms exhibited significant mortality during the adult stage. During the 63 day period over which the experiment was conducted, one WB pair and 50 percent of the CI pairs produced egg cases. None of the CI/EB or EB pairs reproduced. The results of this study indicate that the level of contamination affecting juvenile growth in *Neanthes* is similar to the level that affects reproductive success.

INTRODUCTION

A sublethal sediment bioassay, using the juvenile stage of *Neanthes* has been developed and tested with Puget Sound sediments. Toxicity was determined by measuring differences in worm biomass and survival relative to a control following a 20-day exposure period. The sublethal bioassay is primarily designed to determine the effect of exposure to contaminated sediment on the growth of the juvenile life history stages of *Neanthes*. One approach to assessing the utility of growth as a sublethal measure of organism health is to evaluate the relationship in reduction in growth of the juvenile stage and eventual changes in other ecologically important endpoints such as reproduction. Changes in growth rate (e.g., ability to reach a specific life stage at a given time and within a range of size) can play a significant role in determining the reproductive success of individual organisms and populations. This study was conducted to determine the relationship between juvenile growth in *Neanthes* and reproductive success following exposure to contaminated sediments.

METHODS

The long-term experiment was conducted with sediments collected from Elliot Bay (EB), and Carr Inlet (CI) in Puget Sound, Washington State, U.S.A. The control sediment was obtained from West Beach (WB), a relatively uncontaminated site on Widbey Island, Washington State. In addition to the field collected sediments, a 50/50 mixture (by wet weight) of EB and CI sediments (CI/EB) was included in the experiment to provide a gradient in sediment chemical contamination between the reference (CI) and contaminated (EB) sediments.

The long-term exposure experiment was divided into two phases, which were initiated simultaneously. The first phase of the experiment determined the growth response of *Neanthes* juveniles exposed to a 2 cm layer of test sediment for 10, 15, 20, and 25 days. The protocol described by Johns et al. (1990) was followed except that three replicates from each sediment treatment were taken at the four sampling periods to determine juvenile biomass and survival. The growth response following each exposure period was determined by measuring the increase in biomass of individual worms dried to a constant weight.

The second phase of the experiment determined the effects of sediment exposure on reproductive success. Juvenile *Neanthes* were exposed to the sediment treatments until reaching sexual maturity employing the test protocol described by Johns et al. (1990). At sexual maturity, surviving worms from each treatment replicate were collected and ten worm pairs were selected for each treatment. Each worm pair was placed in a 3 - 4 mm layer of test sediment for the remainder of the experiment. Following 63 days of exposure the experiment was terminated. Response criteria used to evaluate reproductive success were adult worm survival, adult worm size (as dry weight), time to sexual maturity, time to deposition of an egg case, and the relative number of eggs in surviving females.

RESULTS AND DISCUSSION

In the first phase of the long-term experiment, survival was greater than 93 percent in all four sediment treatments. Individual biomass increased with increasing exposure in all treatments with the greatest increase in biomass occurring between days 10 and 20 (Figure 1). A graded response in growth was observed at the end of the 25-day exposure period, with the highest average individual biomass reported for worms from the West Beach and Carr Inlet treatments, followed by worms from the CI/EB treatment. The lowest biomass was observed for worms from the EB treatment. The pattern of growth in worms from the CI/EB treatment was similar to that observed for worms from the EB treatment until day 20. Between day 20 and 25, however, a significant increase in biomass was observed in worms from the CI/EB treatment relative to worms from the EB treatment. Worms maintained in the EB treatment did not exhibit a significant increase in biomass during the 25 day exposure period. In summary, as the amount of contamination in the sediment (determined by the EB fraction) was increased, biomass decreased to the point of almost zero growth in the most contaminated sediment (EB).

A gradation in response was also observed in the response criteria used to assess reproductive success (Table 1). Worms exposed to WB and CI treatments developed to sexual maturity (between days 20 and 25) before worms exposed to the EB and CI/EB treatments (between days 26 and 32). Survival of the adult worm pairs was high (100 percent) in all treatments except the EB treatment. In the EB treatment, only 40 percent of the males and 50 percent of the females were surviving at the termination of the experiment (Table 1). In all four treatments the females were larger than males. Male worms from the WB, CI, and CI/EB treatments were significantly larger than males collected from the EB treatment (Table 1). Females maintained in the WB, CI, and CI/EB treatments were also significantly larger than females collected from the EB treatment (Table 1). At the termination of the experiment, 10 percent of the worm pairs from the WB treatment and 50 percent of the worm pairs from the CI treatment had reproduced. No egg mass was observed in any of the worm pairs from the CI/EB and EB treatments. Surviving females from the WB, CI and CI/EB treatments exhibited a greater relative egg density in the body cavity than did females maintained in the EB treatment. The relative proportion of the body cavity filled with eggs for the three treatments ranged from 94 to 100 percent, while only 58 percent of the body cavity of females from the EB treatment had eggs (Table 1). As with juvenile growth, reproductive success in *Neanthes* appears to be affected by an exposure to both the EB and CI/EB treatments.

The results of this study indicate that a level of sediment contamination causing significant effects on juvenile growth (i.e., exposure to EB sediment) also affected reproductive success in adult *Neanthes*. However, at a lower level of contamination causing equivocal effects on juvenile worm growth (i.e., exposure to CI/EB sediment), there still were clear indications of delays in adult reproduction.

ACKNOWLEDGEMENTS

This study was funded by the U.S. Environmental Protection Agency (EPA) Region 10, Office of Puget Sound, on contract to PTI Environmental Services under the direction of Dr. Thomas C. Ginn.

Research was supervised by Dr. D. Michael Johns of PTI Environmental Services at the E.V.S. Consultants' (E.V.S.) laboratory in North Vancouver, B.C. with the assistance of Dr. Peter Chapman and his staff. *Neanthes* juveniles used in this work were provided by Dr. Donald Reish (California State University, Long Beach).

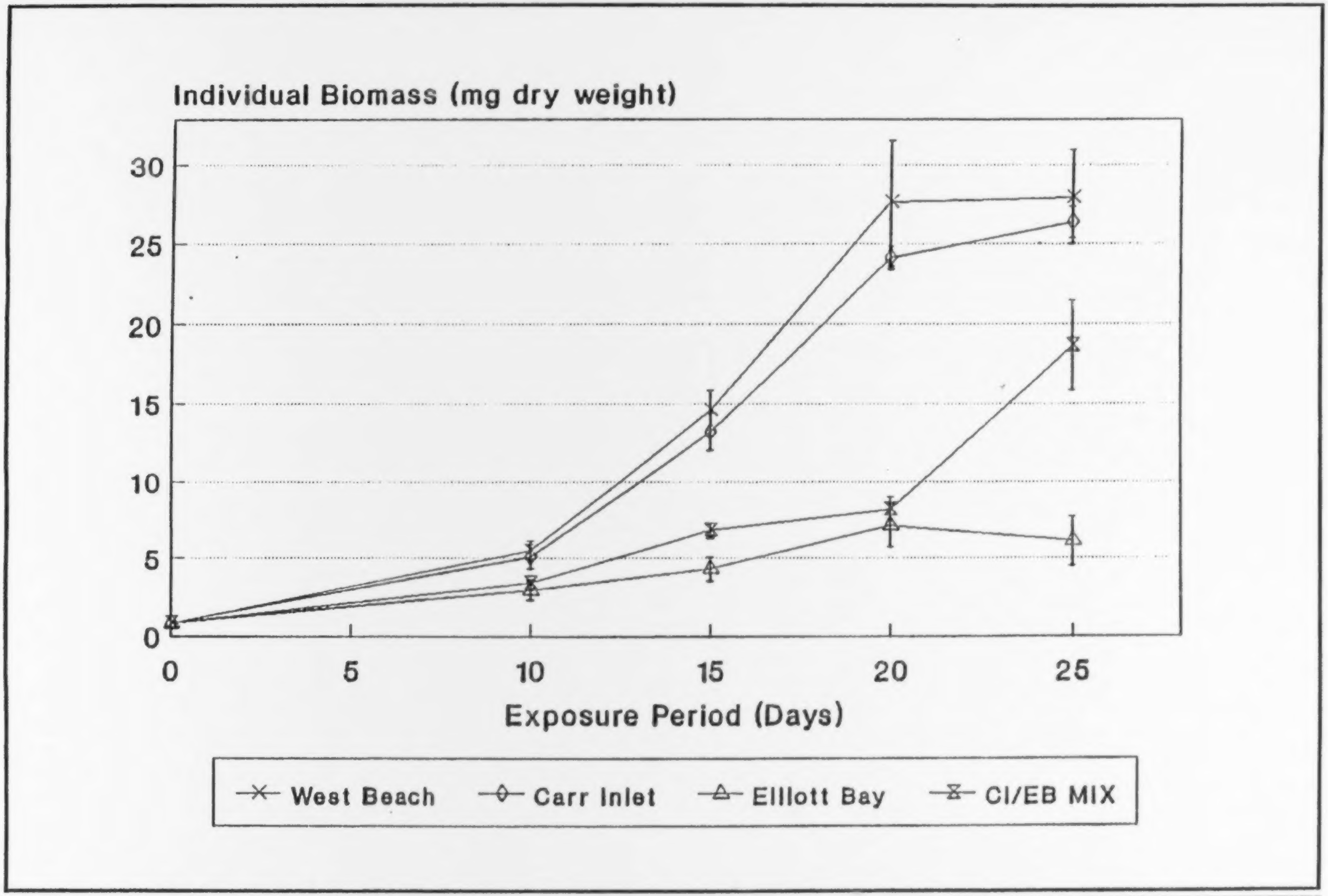

Figure 1. Growth rates and biomass of juvenile worms exposed to Puget Sound sediments. Data are presented as mean ± standard error.

TABLE 1. CHARACTERISTICS OF REPRODUCTIVE SUCCESS IN NEANTHES EXPOSED TO DIFFERENT SEDIMENTS

Sediment Type	Time to Sexual Maturity (days)	Time to Egg Laying (days)	Percent Females Laying Eggs[a]	Percent Male Survival[a]	Percent Female Survival[a]	Dry Weight Surviving Males[a]	Dry Weight Surviving Females[a]	Proportion of Body with Eggs
West Beach	25	52	10	100	100	37.9 ± 2.5	92.5 ± 10.1	0.94
Carr Inlet	25	58	50	100	100	37.0 ± 2.8	93.4 ± 8.9	1.00
Carr Inlet/ Elliot Bay	32	--	0	100	100	27.9 ± 1.7	80.3 ± 7.6	0.95
Elliot Bay	32	--	0	40	50	11.7 ± 2.9	25.8 ± 3.8	0.58

[a] Following 63-day exposure.

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A SUBLETHAL MARINE SEDIMENT BIOASSAY USING THE OPHELIID POLYCHAETE *ARMANDIA BREVIS*

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Numerous bioassay organisms have been tested in an attempt to assess the toxicity of marine sediments, but most available tests suffer from drawbacks which limit their applicability. Limitations in grain size tolerance or availability of the test organism are common drawbacks of many current bioassays. Additionally, most commonly used sediment bioassays measure lethality of the organism as the only effect measured.

Recent emphasis on sublethal measures of sediment toxicity such as relative growth and reproductive success has focused renewed interest in some polychaete worm species. Growth and repoduction are integrated measures of an animals overall health and may provide a more sensitive means of measuring sediment toxicity than bioassays based solely on lethal effects to the test organism.

Armandia brevis is a subsurface deposit feeding Opheliid polychaete with an opportunistic life history. Juvenile worms of approximately 5 mg wet weight are used for the growth assay and worms of this size are available year round at numerous collection sites in Puget Sound. In 20 day bioassays under optimal conditions, worms increased three fold in wet weight when exposed to uncontaminated sediment and fed a diet of commercial tropical fish food flakes. This scope for growth provides a sensative means of discriminating sediment contamination.

The influence of salinity, size of animal, temperature, and sediment grain size on *A. brevis* growth was evaluated and optimum growth conditions were determined. Optimum conditions for growth of *A. brevis* in this bioassay include a temperature of 12±1°C, salinity greater than 30ppt and the use of small (5mg) juvenile animals. Sediment grain size ranging from 0.35 to 52% clay-silt and total volitile solids ranging from 1.09 to 5.40% had little discernable effect on *A. brevis* growth. Growth of

1.09 to 5.40% had no discernable effect on *A. brevis* growth. Growth of *A. brevis* on sediments that were moderately to heavily contaminated with toxicants was significantly depressed relative to growth of *A. brevis* on minimally contaminated sediments, even though mortality of *A. brevis* was apparent only in the most contaminated sediment. Application of this subleathal growth bioassay to evaluate the toxicity of marine sediments may prove useful compared to existing mortality based bioassays.

COMPARATIVE TOXICITY OF TRIBUTYLTIN TO TWO SPECIES OF INFAUNAL AMPHIPODS.

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Recently developed sediment bioassay protocols indicate that amphipod species should be selected based on their sensitivity to toxicants. Several species are listed as possible candidates for testing but few intercomparisons have been performed to determine their relative sensitivity to various toxicants. One might be tempted to expect that similar species (e.g. gammaridean amphipods) respond similarly to a given toxicant, but there is no support for this assumption.

The relative sensitivity of tributyltin (TBT) to two cooccurring infaunal amphipods species (*Rhepoxynius abronius* and *Eohaustorius washingtonianus*) was determined. The experiments were conducted as water-only experiments in order to eliminate any response to physical/chemical parameters and to reduce the influence of environmental parameters on the toxicant's bioavailability.

Comparison of each species' concentration-dependent response determined that *R. abronius* was about 30 to 100 times more tolerant of TBT than *E. washingtonianus*. The 4 day and 10 day LC50 was 138 (± 33) and 29 (± 6) for *R. abronius* and 4.7 (± 2.2) and 0.3 (± 0.2) for *E. washingtonianus*. The decrease from day 4 to 10 was substantial, indicating that tributyltin is a slow-acting toxicant. The survivors of individuals in these experiments also exhibited differences in a sublethal response (reburial behavior) to TBT which may be related to their tolerance of the toxicant.

Body burden analyses determined that the bioconcentration factor (BCF) for *R. abronius* was in the order of 2000 to 3000 (based on dry weight), which is an order of magnitude lower than that for *E. washingtonianus*. Because the BCFs were so different, we decided to examine their metabolic capacity.

It is hypothesized that metabolic differences may explain, in part, the varied response of these species to TBT exposure. The work of Reichert et al. (1985) determined that *R. abronius* produced more than twice as much metabolites of benzo(a)pyrene than *E. washingtonianus* when exposed for 7 days. Hence, the more efficient metabolic system of *R. abronius* probably allowed a higher throughput of TBT, causing the lowered TBT bioconcentration factor and higher tolerance to this toxicant.

When the percent mortality was plotted against tissue concentration, it was discovered that *E. washingtonianus* exhibited a similar response when the whole tissue levels of TBT were about 20% of those observed in *R. abronius*. Significant mortality in *E. washingtonianus* was observed when the whole tissue concentration was above 10 ppm (dry weight). It was expected that these dose-response curves would be similar. The difference in the mortality response for a given whole tissue body burden may be explained by differences in total lipids or differential partitioning of the toxicant in the various organs. Because TBT is lipophilic, a larger amount of total lipids in one species may sequestor more of the toxicant and thus lower toxicity.

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EFFECTS OF OILED SEDIMENT ON THE EMERGENCE BEHAVIOUR OF CAPELIN (*MALLOTUS VILLOSUS*) LARVAE

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ABSTRACT

Capelin spawn in intertidal or subtidal sediments. The embryos incubate within the sediments for 2-3 weeks, and larvae emerge during onshore winds. The water mass associated with these onshore winds is warm, food-rich, and predator-poor. Synchronization of emergence with onshore winds is an important adaptive behaviour that may determine subsequent year-class strength.

A flow-through bioassay was developed which assessed the effects of oiled sediment on the emergence of capelin larvae. Capelin were incubated in oiled gravel (25-400 ppm total hydrocarbons) from the blastula stage to larval emergence. The test system reproduced the natural intertidal water temperature changes associated with changes in wind direction. Larvae from oiled sediment emerged earlier, and continued to emerge over a longer interval, than did control larvae. Emergence behaviour was a sensitive measure of effects, but those effects were not negative. Instead, accelerated and sustained emergence represents an adaptive and flexible response to stresses such as pollution.

INTRODUCTION

Capelin spawn in intertidal and subtidal areas along the North Atlantic coast. The adhesive eggs incubate in beach gravel for 2-3 weeks. After hatch, larvae may remain in the gravel for a further few days, eventually emerging during periods of onshore winds. Onshore winds bring in a warm water mass rich in food but with few predators. The emergence interval is critical, because year class strength of the harvested adult population may be determined during this interval (Leggett et al. 1984). Capelin embryos and larvae would be particularly vulnerable to the effects of a nearshore oil spill, as the oil would become stranded on the spawning beaches or concentrated in inshore larval rearing areas.

This paper provides the results of a sediment bioassay investigating the effects of oiled sediment on the emergence of capelin larvae. Experiments were conducted in Bryant's Cove, Conception Bay, Newfoundland, a major and extensively studied capelin spawning area (see Frank and Leggett, 1981*a,b* for a description of the site). Thus, there was a unique opportunity to use meaningful response measures, determined by previous research, in an experiment assessing the effects of a pollutant (oil) that was of concern both locally and globally.

METHODS AND MATERIALS

Emergence behaviour was used as a response variable in a bioassay examining the effects of Hibernia crude oil on capelin embryos and larvae. We developed a flow-through rearing system which reproduced the natural temperature variability associated with changing wind directions and subsequent water mass movement (Fig. 1, 2). Six nominal concentrations of oiled sediment were used: 0, 25, 50, 100, 200, 400 ppm. Four aquaria were subdivided into six leak-proof compartments each; the six concentrations were randomly assigned to the compartments. The experimental design was therefore a randomized complete block. The aquaria were held in a water bath supplied with a constant flow of water pumped from approximately 100 m offshore. Each individual compartment was also supplied with a constant flow (3.6 L/h) of the same water (filtered).

Embryos were obtained from the beach at Bryant's Cove shortly after the first major spawning of 1988. Egg-bearing gravel was collected from the beach, rinsed, then placed in the aquarium compartments (Fig. 1). Egg numbers were 10,000-30,000 per compartment, providing a density of 9-32 eggs/cm^3. Natural egg densities on the spawning beach are 10-140 eggs/cm^3 (Frank and Leggett, 1981*b*). Embryos were reared in the oiled sediment from the early blastula stage to larval emergence. Emerged larvae were collected twice daily over a 10-day interval.

Concentrations of oil in the sediment and interstitial water of one aquarium were monitored throughout the experiment. Gravel samples of approximately 50-100 mL were removed with a scoop; no attempt was made to sample discrete depths. As a result, concentrations, including nominal concentrations, refer to the entire 6-cm layer of gravel, and probably underestimate concentrations in the initial 2-cm oiled layer. However, the concentrations of hydrocarbons in the interstitial water were presumably homogeneous with respect to depth, and it is the interstitial concentrations which would produce effects. Total hydrocarbon concentrations of sediment and water were measured by ultraviolet spectrophotometry, concentrations of individual compounds were measured by gas chromatography.

RESULTS

During the experiment, concentrations of oil (expressed as total hydrocarbons) in the sediments declined by 0-70%, with the greatest reductions occurring at the highest concentrations (Fig. 3). Total hydrocarbon concentrations in the interstitial water declined by approximately 80-90% (Fig. 3). Concentrations of naphthalenes in the gravel decreased by 50-70%; concentrations of C8-C26 n-alkanes decreased by 95%. (These decreases in naphthalenes and alkanes were measured only at the highest concentration tested, and were presumably lower at lower concentrations.)

Percent hatch ranged from 87-96% in all treatments, and was not related to oil concentration (Fig. 4). Sixty-six percent of control larvae emerged; significantly more (77-82%) exposed larvae emerged (Fig. 4). Larvae exposed to all concentrations of oiled sediment emerged earlier, and continued to emerge over a longer interval, than did control larvae (Fig. 5). Exposed larvae did not emerge at inappropriate temperatures associated with offshore winds, but appeared to respond more quickly to the higher temperatures associated with onshore winds.

Even though they emerged earlier, exposed larvae developed at a slower rate than did control larvae (Fig. 6). These effects on developmental rate are probably biologically insignificant, as the mean length of exposed larvae was only 1% shorter than that of control larvae collected at the same time. Exposure also appeared to affect the diurnal pattern of emergence. Control larvae emerged primarily at night, whereas exposed larvae were almost as likely to emerge during the day as at night (Fig. 5).

DISCUSSION

We conclude that realistic exposure conditions during the embryonic period are unlikely to negatively effect emergence behaviour of capelin larvae. Emergence behaviour was a sensitive measure of effects, but the effects that occurred were not negative. Accelerated and sustained emergence of exposed larvae probably represents an adaptive and flexible response to stresses such as hydrocarbon contamination.

We are concerned about the delayed effects of embryonic exposure on post-emergent larvae, which we have observed in other experiments (Paine et al., in review). Lipophilic compounds such as hydrocarbons may be preferentially stored in the yolk or lipid globule of embryos and larvae, and may not be released into the larval body until yolk reserves are almost entirely depleted. At that time, the larvae would experience a concentrated "slug" of contaminants.

Emergence behaviour is a biologically meaningful and sensitive response variable which should be included in considerations of sediment toxicity for species with reproductive styles similar to those of capelin. Effects on larvae should be monitored past the time of emergence to yolk exhaustion. Finally, sediment bioassays conducted on embryos and non-feeding larvae can be used to separate the effects of uptake of contaminants from interstitial water from any effects associated with ingestion of contaminated sediment particles.

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Paine, M.D., W.C. Leggett, J.K. McRuer, and K.T. Frank. In review. Effects of Hibernia crude oil on capelin (*Mallotus villosus*) embryos and larvae. I. Effects on larvae. (Submitted to Mar. Environ. Res.).

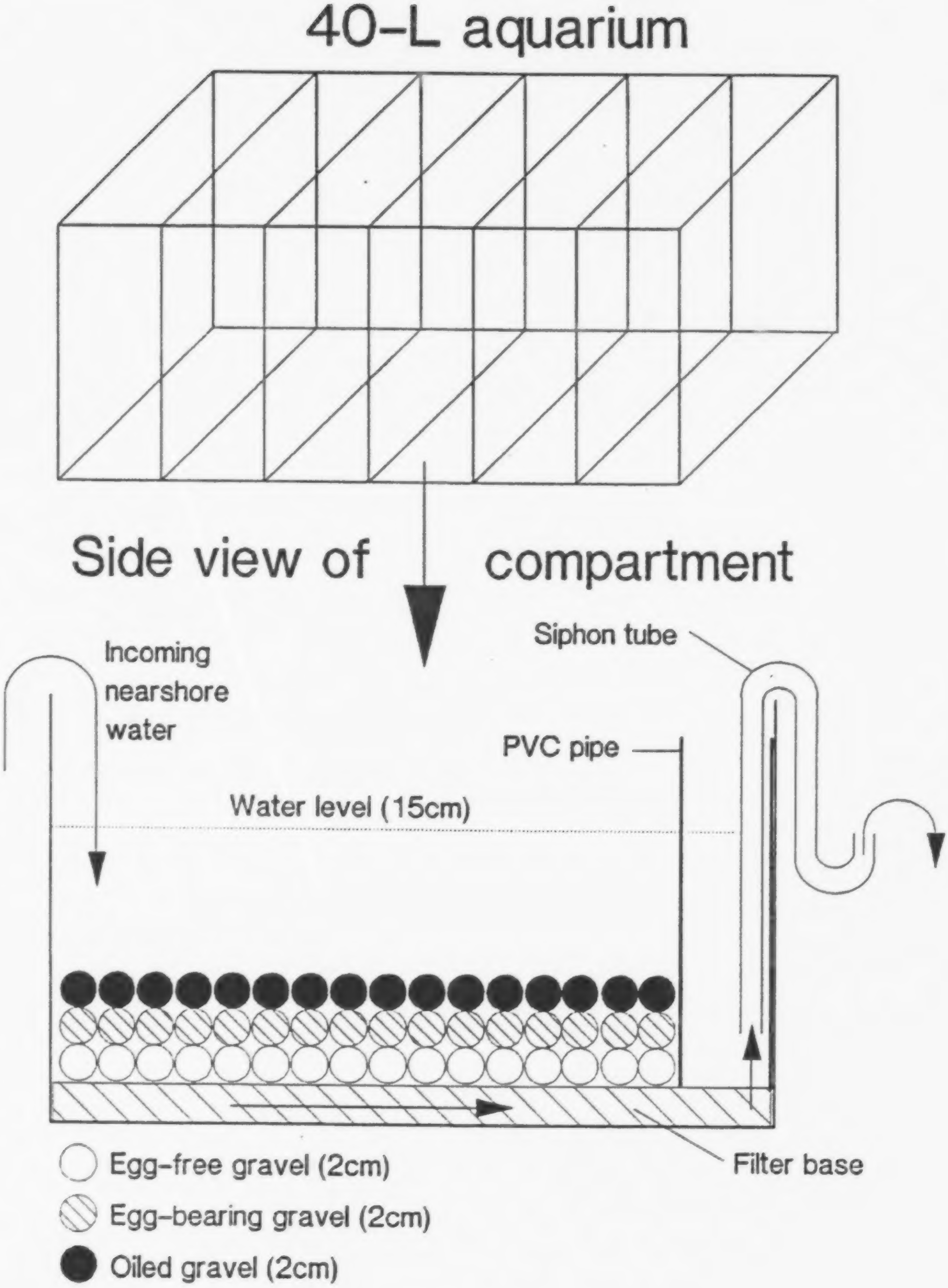
40-L aquarium
Side view of compartment
Incoming nearshore water
Siphon tube
PVC pipe
Water level (15cm)
Filter base
Egg-free gravel (2cm)
Egg-bearing gravel (2cm)
Oiled gravel (2cm)

Figure 1. Design of flow-through exposure system. Arrows in side view of compartment indicate direction of water flow.

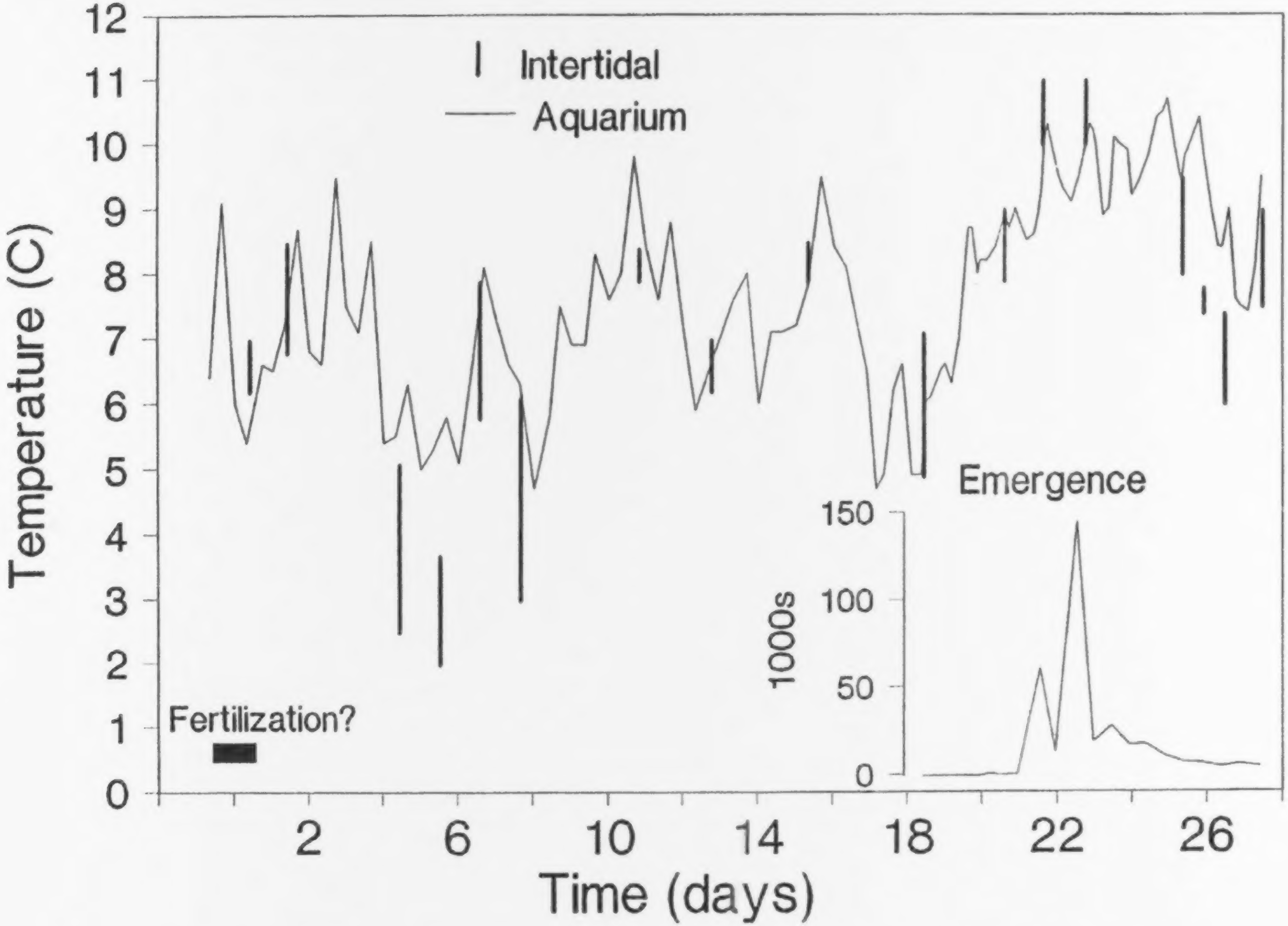

Figure 2. Temperatures in the experimental aquaria and in the intertidal zone of Bryant's Cove, Newfoundland. The high temperatures corresponded with onshore winds; lower temperatures corresponded with offshore winds. The small-scale fluctuations resulted from daily temperature cycles.

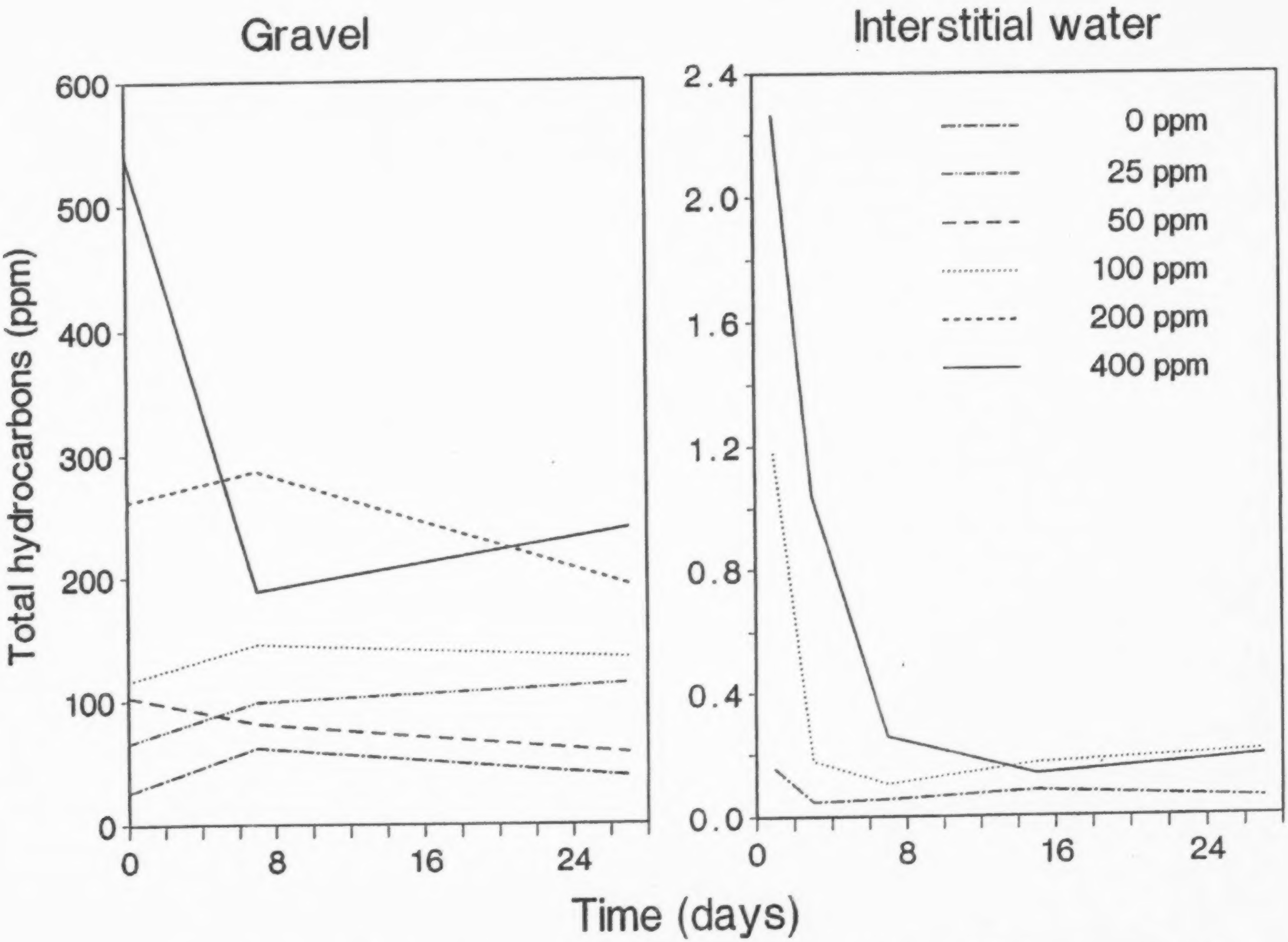

Figure 3. Total hydrocarbon concentrations in the gravel and interstitial water. Note the convergence of concentrations despite the large differences in initial concentrations, and note the background levels present in controls.

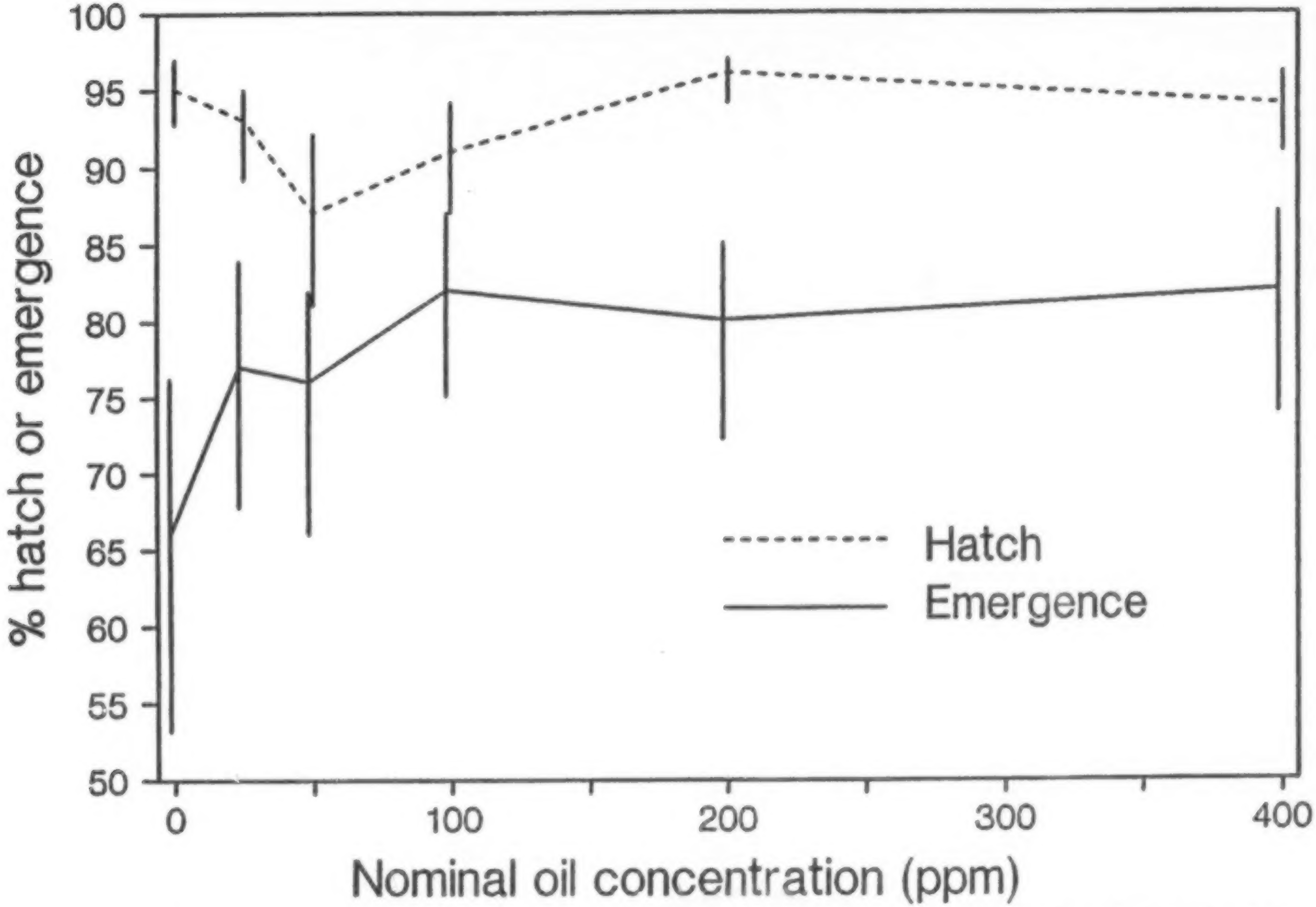

Figure 4. The effects of oiled sediment on hatch and emergence. Vertical bars are ± 2 SE. Values for hatch are percentages of the number of eggs stocked; values for emergence are percentages of the number of larvae. Values were back-transformed from log (100-X).

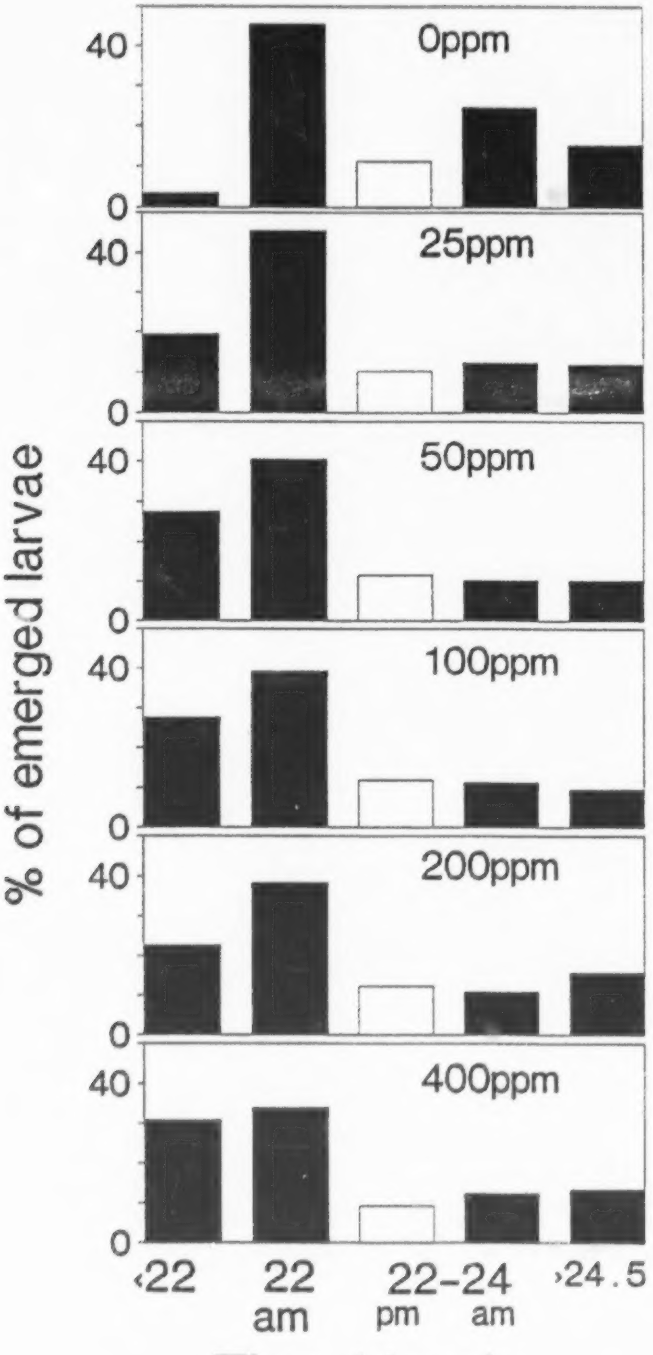

Figure 5. Effects of oiled sediment on emergence patterns. Larvae emerging at night were collected in the morning (a.m.); larvae emerging during the day were collected at night (p.m.). Note the pronounced day-night difference for controls on days 22-24, which was not evident for exposed larvae.

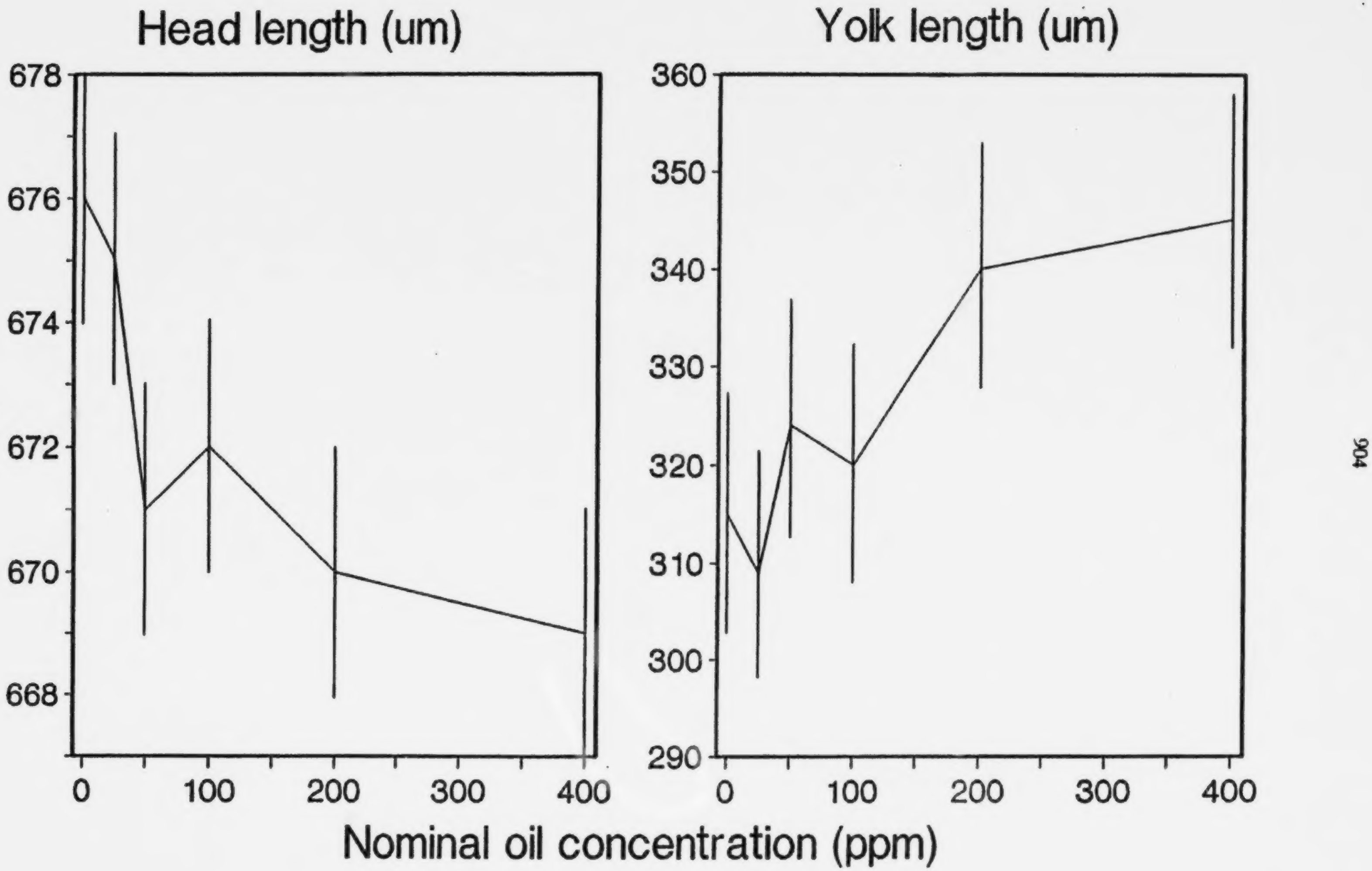

Figure 6. Effects of oiled sediment on head and yolk length. The lengths given are means of the means from twice-daily collections, and are thus unaffected by any differences in timing of emergence. Vertical bars are ±2 SE; values for yolk length were back-transformed from log (X). Note that exposed larvae developed more slowly (smaller heads, larger yolks) than did control larvae.

ACUTE SEDIMENT TOXICITY ASSESSMENT: A NEW TEST FOR THE UNITED KINGDOM. B.D. Roddie and T. Kedwards, WRc, Medmenham Laboratory, Po Box 16, Marlow, Bucks. SL7 2HD, United Kingdom (491 571531)

At present in the UK there are no recognised toxicity criteria for sediment quality assessment. WRc is developing, on behalf of the National Rivers Authority , lethal and sublethal toxicity test methods for marine, estuarine and freshwater sediments. A recent seagoing Biological Effects workshop organised under the auspices of ICES and the IOC provided the opportunity to apply our provisional lethal marine test methods to sediments from two distinct contamination gradients in the North Sea. The results of these tests will eventually be evaluated in conjunction with those of tests conducted by four other laboratories on the same material and with synoptically-acquired benthic and chemical data. WRc conducted 10-day solid-phase tests on samples collected in triplicate from each of 16 stations. Test endpoints were survival and immobilisation in the intertidal amphipod Corophium volutator. Preliminary analysis of the results demonstrated good quality control and responses which indicated that the test was able to detect previously-identified contamination gradients. Laboratory-level replication underlined large differences in the toxicity of spatial replicates at some stations.

A New Microbial Bioassay for Screening of Sediment Toxicity

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A methodology for a standardized, microbial bioassay has been developed and adapted to determine toxicity in organic extracts from marine sediment samples. This test is based on *de novo* inhibition of the induced enzyme β galactosidase in an engineered strain of *E. coli*. Sensitivity is enhanced by stressing the bacteria and then combining their recovery period with the toxicant exposure phase. The bacteria's recovery from stress, under influence of potentially toxic samples, determines their capacity to synthesize β galactosidase. Enzyme activity is readily detected by chromogen reaction. Results are read either visually or by spectrophotometry. Since the exposure period is several times longer than the generation time of *E. coli*, this single endpoint actually integrates measures of survival and reproductive success into one parameter. The bioassay is conducted in a few hours in 96-well microtitration (ELISA) plates, utilizing very small sample volumes. Over 100 samples can be tested in one day. This new tool offers a high-volume, low-cost alternative for the rapid screening of sediment toxicity.

Marine sediments are known to absorb numerous persistent chemicals to many times higher concentrations than their water column levels. Sediments have been identified as the primary repository for numerous toxic compounds, including trace metals, chlorinated hydrocarbons, PAHs, and PCBs. Many of the contaminants present in marine sediment are also carcinogenic in some organisms. Detailed chemical analyses of urban sediments have identified over 500 aromatic hydrocarbon compounds (Malins *et al*. 1982). The biological impact of highly complex mixtures of contaminants, with a sediment matrix effect included, is essentially impossible to predict given chemistry results alone. Hence the biological assessment of toxicity of contaminated sediments has recently become a major concern and emphasis.

Bioassays have proven to be useful procedures for the assessment of biological impacts of contaminated sediments. Two principal types of tests have been employed--those using eukaryotic organisms and those which expose bacteria. In practice, bioassays using eukaryotic species have most commonly measured acute mortality as an endpoint (Buchman In press). This approach is not without its drawbacks, however. To obtain results, these tests typically require exposure periods of at least a couple days, and sometimes up to weeks. Also, the many logistical problems associated with the handling and testing of higher organisms, plus the more pronounced genetic differences naturally present among large, individual organisms (*e.g.*, fish), adds to the variability of results.

Microbial tests, the other major type of bioassays, have been used to study environmental toxicity for some time (Bitton and Dutka 1989). However, microbial tests only recently have been applied to investigations of sediment toxicity. These bioassays developed partly in response to fill the gap for sensitive, rapid, and inexpensive tests of toxicity (Bitton and Dutka 1989). In contrast to most eukaryotic tests, microbial bioassays can test an extremely large population in a relatively short period of time. Microbial populations have relatively short life cycles and respond quickly to environmental changes. They are fairly stable and can be maintained and cultured in a laboratory at low cost.

One microbial assay of toxicity that has been employed widely measures reduction in light production of the luminous bacteria ***Photobacterium phosphoreum*** as an indication of the health of the bacteria's electron transfer system. Performance of this test has compared favorably with results from other bioassays (Bulich and Isenberg 1981). Though originally developed for testing of aqueous solutions, methods for testing of marine sediment samples have been developed (Schiewe *et al.* 1985) and its use in marine assessments has been increasing. Results from these marine studies have also compared favorably with results from other assessment techniques (Anderson *et al.* 1988; PTI 1988).

This paper presents a methodology for the application of new microbial assay to the determination of marine sediment toxicity--the Toxi-Chromotest™. Like the *Photobacterium* bioassay, this test and its companion test of mutagenicity (see this volume) were originally developed for use with aqueous solutions (Reinhartz *et al.* 1987). These tests have been used to assess the toxicity and mutagenicity of drugs and foodstuffs, but have only recently been employed in environmental studies.

The Toxi-Chromotest™ is based on the ability of toxicants to penetrate through the cell wall of a highly permeable, mutant strain of *Escherichia coli* and inhibit *de novo* synthesis of an inducible enzyme, β-galactosidase. Sensitivity of the test is enhanced by pre-stressing the bacteria and then combining the bacteria's recovery phase and enzyme induction phase with the toxicant exposure period. Activity of the induced β-galactosidase is detected by reaction with a chromogenic substrate. Results can be read qualitatively with the naked eye or quantitated using microtitration plate spectrophotometers (ELISA readers). Because of the speed with which results may be obtained with Toxi-Chromotest™, this bioassay shows promise as an extremely rapid screening tool.

To evaluate the performance of the Toxi-Chromotest™ on environmental samples, bioassays of organic extracts of sediment contaminated from an oil spill were conducted. To evaluate the feasibility of the Toxi-Chromotest™ as a real-time screening tool, tests were conducted daily onboard ship. Samples at each site were analyzed the same day as collected, and final results were reported within 24 hours.

Materials and Methods

Sediments used for this evaluation were collected from the intertidal zone to depths of 100 meters and were contaminated to varying degrees with crude oil. Samples were collected at sites where the likelihood of any other form of anthropogenic contamination was negligible. Surficial sediment samples were collected from the intertidal zone by hand using solvent-rinsed scoops and placed in solvent-rinsed glass jars which were sealed with Teflon sheeting. Samples from moderate depths were collected by SCUBA divers in Whirl-Pak™ bags. Deep sediment was collected with a Van Veen grab and samples were taken from the top 2 cm using a solvent-rinsed scoop and transferred to solvent-rinsed glass jars sealed with Teflon sheeting.

Sediments were extracted within several hours of collection using a modification from Parsons *et al.* (1984). The choice of method was partially dictated by expediency and ease of onboard operation. Samples from 1 to 5 grams were mixed with an equal weight of anhydrous sodium sulfate plus methylene chloride at a rate of 1 mL per gram and then sonicated for 30-60 seconds. This procedure was repeated twice more, with the extracts combined and the total volume brought up to 25 mL. This methylene chloride extract was then exchanged to dimethyl sulfoxide (DMSO). A 5 mL split of the methylene chloride extract was concentrated to approximately 0.5 mL in glass centrifuge tubes in a water bath at 40°C. A 100 µL of DMSO was then added, and the mixture again concentrated to remove the remaining methylene chloride. The resulting DMSO extract was used in bioassays. Extraction blanks without the sediment were prepared by an identical procedure.

Toxi-Chromotest™ bioassays were conducted in sterile, disposable, 96-well ELISA microtitration plates (8 rows by 12 columns). Two or three plates of samples (up to 22 samples) were run during each batch. Each sample was run in duplicate dilution series. Each batch included a positive control and solvent blank. Negative controls were run for every sample.

A rough mutant of *E. coli*, K12 OR85, was supplied by Orgenics in lyophilized form. The bacteria were rehydrated for 15 minutes at room temperature in 10 mL of growth medium (1% Bacto Tryptone, 0.5% Bacto yeast extract, 1% sodium chloride). This resulted in a final suspension of approximately 2.5 x10^8 cells per mL (Reinhartz *et al.* 1987). A 1 mL portion of this suspension was then mixed into 6.5 mL of reaction mixture containing 1.2% sodium chloride, 50 (millimolar) mM potassium chloride, 20 mM sodium phosphate buffer, 0.3% Bacto tryptone, 0.15% Bacto yeast extract, and 0.014% isopropyl β-D-thiogalactopyranoside (IPTG). IPTG is a specific inducer for the β-galactosidase enzyme. Other factors of the reaction mixture are required for recovery of the bacteria from their stressed condition.

While the bacteria were rehydrating, ELISA plates were prepared. Plates were arranged to accommodate six samples on the master plate and eight samples on subsequent plates. One set of wells, which served as a machine blank, received 100 µL of standard diluent (5% DMSO in saline) and 100 µL of the reaction mixture without bacteria. A second set of wells contained a serial dilution of the standard toxicant, 4 $^{\mu g}/_{mL}$ HgCl. Duplicate solvent blanks were run in serial dilution in the third and fourth sets. Remaining sets were divided among test samples. Each test well received 100 µl each of the diluent and bacterial reaction mixture. Serial dilutions were prepared for each set by transferring solution with a calibrated, digital multichannel pipette. The last row of wells in every test set received distilled water and served as negative controls. After samples were dispensed, plates were incubated for 2 hours at 37°C.

Following incubation, 100 µL of chromogenic mixture were added to each well. The chromogenic mixture contains lysing agents (detergents and solvents) in a pH 9.0 Tris buffer system and bromo chloro indoxyl β-D-galactoside (BCIG). BCIG develops into a deep blue color in healthy, reproducing bacterial suspensions. No color development indicates an inviable suspension.) The plates were then returned to the incubator for an additional 2 hours after which time the optical density (OD) at 615 nm was read in an ELISA spectrophotometer.

Toxicity for each well was defined and calculated by the following equation

(1) $\% \text{ Toxicity} = (1 - OD_{\text{test well}} / \text{Average } OD_{\text{negative control wells}}) \times 100$

Toxicities from the dilution series were used to determine an EC_{50} for each replicate of each sample. The EC_{50} was defined as the concentration of sediment extract in mg sediment per mL solvent which resulted in 50% toxicity. This value was obtained by regression analysis.

The presence of oil in sediment extracts was analyzed by florescence at 310 nm excitation and 370 nm emission. The instrument was blanked with methylene chloride, and calibrated against standard dilutions of crude oil extracts for each sample run.

All solvents were HPLC grade. Standard diluent was prepared with Millipore ion-exchange purified water. All other reagents were supplied in kit form from Orgenics.

Results

The Toxi-Chromotest™ bioassay is based on the principle that inducible metabolic systems are sensitive to toxicants. Furthermore, it was presumed that sensitivity to toxicants would be greater than to carrier solvents. This assumption was tested by conducting bioassays using organic extracts of contaminated sediments exchanged into DMSO. An example of results for toxicity in duplicate dilution gradients of a sample are shown in Figure 1. Toxicity (Y axis) is expressed as a percentage according to equation (1). The sediment extract concentration (X axis) is expressed in terms of mg of sediment per mL of solvent and represents an effective concentration. Replicate assays of samples showed good agreement; the median coefficient of variation between duplicate determinations of EC_{50} among all samples tested (n=74) was only 5%. This compares quite favorably with numerous other sediment bioassays (Long and Buchman 1989), and indicates a high degree of precision. Low coefficients of variation also suggest a high statistical power for discriminating between sites (Long and Buchman 1989). The curves of Figure 1 also demonstrate that the Toxi-Chromotest™ bioassay is dose responsive to toxicant concentrations. Because results from an entire dilution gradient are used to derive the EC_{50} deviations in performance of a single well has little effect on EC_{50} values.

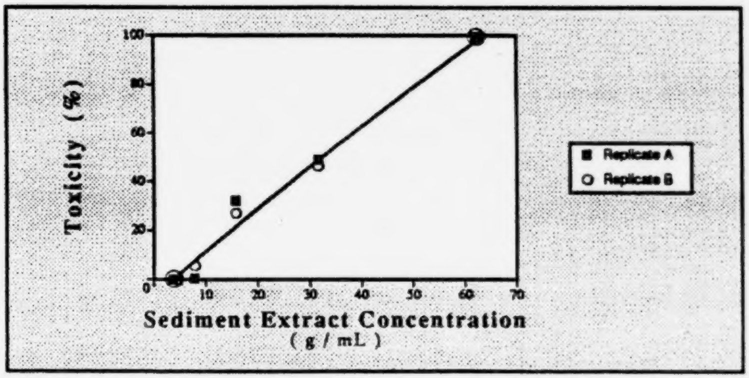

Fig. 1. Results of Toxi-Chromotest™ bioassay in duplicate analyses of a sample. EC_{50} value is derived from duplicate results by regression analysis, and is expressed in terms of grams sediment per mL of solvent extract. Toxicity is expressed as a percentage according to Equation (1).

The results from assays of all samples indicate a broad range in response by the Toxi-Chromotest™ bioassay. EC_{50} values ranged from 2.7 to 67.4 $^{mg}/_{mL}$. Performance of the test, relative to degree of contamination, was also quite good. The association between toxicity and the degree of contamination, expressed as crude oil equivalents, for all samples collected from 6-100 meters ($n=51$) is depicted in Figure 2. The Spearman rank correlation between EC_{50} values and crude oil equivalents, at 0.52, was highly significant ($\rho = 0.0001$).

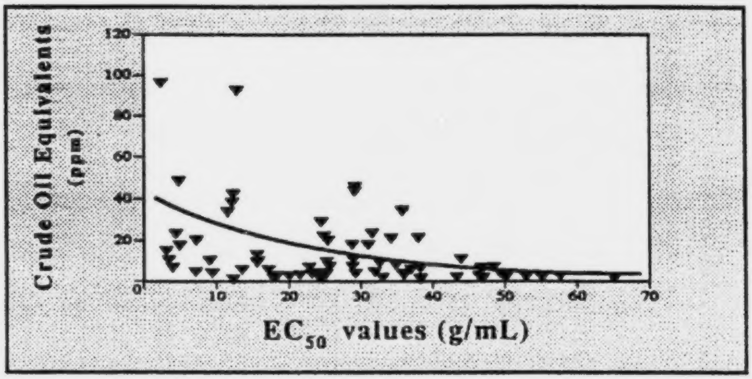

Fig. 2. Association between Toxi-Chromotest™ bioassay results, in terms of EC_{50} values, and crude oil equivalent concentration of sediment parts per million (ppm) as determined by bulk aromatic hydrocarbon fluorescence.

Although the association between contamination and toxicity was statistically significant, the degree of variability increased with increasing toxicity, as evident in Figure 2. Increased variance would statistically reduce the ability to differentiate between sites. There are factors which could explain this trend. For instance, responses to natural biocide substances apparently occurred. Results from samples at one site indicated high toxicity, despite low florescence. (The high toxicity was also verified by independent means.) The presence of a dense mat of decaying woody material and pine cone fragments in the sediment samples would explain these results. High toxicity due to organic compounds produced by conifers and eucalyptus trees has been observed in other studies (Long 1990). Also, the presence of sulfides was noted in many samples. If a residual fraction of sulfides passed through the extraction process, this would result in increased toxicity. Additionally, since toxicity is expressed in terms of mg of sediment per mL of solvent, and a very wide range in grain size among samples was noted, it is anticipated that the relationship between contamination and toxicity would be improved if each EC_{50} was normalized to grain size or organic carbon content. Furthermore, in grossly contaminated samples, not all of the extract obtained was soluble in DMSO. Though toxicity was still indicated, the level of toxicity tended to be lower than bulk fluorescence would predict.

Application of the Toxi-Chromotest™ onboard ship, in real-time fashion, proved this bioassay's worth as an extremely rapid screening tool. Ship operations had little impact on operation of the bioassay. Though the integrity of a very few samples was impacted, these samples were easily and quickly re-analyzed. The limiting factor in performing bioassays was the rate at which sediment samples could be extracted.

Discussion

Although bacterial bioassays have been employed for sometime in the testing of environmental toxicity, their application to marine sediments has been limited. We evaluated the performance of a new bioassay, the Toxi-Chromotest™, and concluded that it has a high degree of potential as a screening tool of general sediment toxicity. Moreover, performance of the test onboard ship, in real-time data generation mode, was found to be feasible and reliable.

The Toxi-Chromotest™ is based on the ability of toxicants to penetrate the cell wall of a permeable strain of *E. coli* and inhibit *de novo* synthesis of an inducible enzyme, β-galactosidase. The basis for the enhanced sensitivity of the test lies in lyophilizing the bacteria and then combining the bacteria's recovery phase and enzyme induction phase with the toxicant exposure period. Lyophilization imparts functional and structural damage requiring recovery processes involving numerous metabolic pathways. Following rehydration, a period of heightened activity occurs (Sinskey and Silverman 1970). This repair period requires functioning protein, RNA, oxidative phosphorylation, and energy metabolism systems (Ray and Speck 1972). Although many aspects of the bacteria's recovery are dependent on a viable electron transport system, inducible enzyme systems have been found to offer a more sensitive assay of toxic action. Reinhartz *et al* (1987) reported induced β-galactosidase activity was 8 times more sensitive to arsenite than a pre-induced, ongoing synthesis, and also 8 times more sensitive than assays of the electron transport system. They also report a lag period of 30 minutes before

E. coli begin to synthesize β-galactosidase (Reinhartz *et al.* 1987), during which fine compounds may impart toxic action upon any of the cells metabolic activities. This results in a lower background activity and increased sensitivity. This lag period of lyophilization recovery is also accompanied by a significant increase in cell membrane permeability. This is especially advantageous since it enables high molecular weight compounds, which would otherwise be blocked in fresh cells, to permeate through membranes (Reinhartz *et al.* 1987).

Since toxicants can interfere not only with the specific enzyme measured but with the bacteria's entire recovery process and subsequent reproduction, the Toxi-Chromotest™ represents a more encompassing, holistic measure of the bacteria's overall condition; and, therefore, a broader assay of general toxicity. The test has been shown to be responsive to aqueous solutions of over a hundred compounds, including trace elements, pesticides, mycotoxins, foodstuffs, and drugs (Bio-Response Systems 1988). As a screening tool, this test offers numerous advantages. Bioassays of well over 100 samples can be conducted in one day at very reasonable costs (estimated at $15 US per sample). Results can be read visually or with widely available, inexpensive spectrophotometers. When results are quantitated by spectrophotometry, the actual physical endpoint measured, optical density, is objective and highly reliable. Availability of bacterial cultures is not limited, but eliminates native population interference. Space requirements (a desktop) and the sample size needed to conduct the bioassay are quite minimal. Logistically, extraction of sediment appears to be the main time- and space-consuming constraint.

Inherent in the organic extraction process is a limitation on the class of compounds which the bioassay will be responsive to–primarily neutral, nonionic organic compounds. The presence of additional classes of compounds cannot be ruled out however (Schiewe *et al.* 1985). Alternative extraction methods could also be developed to incorporate a broader spectrum of compounds in sample extracts. Organic extracts also do offer some advantages. Sample extracts may be archived quite easily. Also, extract fractionation can be performed to identify the fraction which contains the most active toxic agents, and thereby provide indications as to the source of toxicity.

The results presented here demonstrate that the Toxi-Chromotest™ can perform with a high degree of sensitivity, precision, and discriminatory power. This development and field trial of a marine sediment application indicates that this bioassay shows promise for evaluations of marine environmental toxicity. Further evaluations of the bioassay should be conducted with samples from marine environments contaminated by a wide variety of anthropogenic compounds.

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PLATFORM SESSION

Toxicology & Sustainable Development

Chair: D. Vailiela

SUSTAINABLE DEVELOPMENT, ENVIRONMENTAL CAPACITY AND WATER QUALITY STANDARDS: INTRODUCTORY PERSPECTIVES. Barry Sadler, INAWA, Victoria, BC and Patrice LeBlanc, FEARO, Ottawa, ON.

The concept of sustainable development has achieved a high public and political profile in recent years. It provides an over-arching goal and frame of reference that links environmental and economic imperatives and interests. Much work is ongoing to elaborate the policies, principles and procedures for achieving sustainability. This research is being applied to various resource contexts, including aquatic and fisheries management.

In this paper, we explore the policy linkages between sustainable development, environmental capacity and water quality standards. The purpose is to provide a background/introductory perspective on contemporary issues of aquatic toxicity. We intend to argue these four points:

1. environmental capacity is the enabling condition of sustainable development;
2. given present trends, the source and sink functions of most aquatic systems must be maintained at or near present levels;
3. conventional approaches to establishing water quality (or assimilative) standards and undertaking environmental assessment must be revamped; and
4. a greater emphasis should be given to regional thresholds and the cumulative relationship between total stresses and eco-system integrity.

This brings into policy-focus some important scientific and technical issues regarding toxic discharges and their cumulative impact on aquatic and human health.

A TOXICOLOGICAL PERSPECTIVE ON ECOSYSTEM CHARACTERISTICS TO TRACK SUSTAINABLE DEVELOPMENT. ECOSYSTEM HEALTH. VII.

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ABSTRACT

"Ecosystem Health," an emerging science paralleling human and veterinary medicine, has as its goals the systematic diagnosis and treatment of stressed ecosystems. Ecosystems are stressed by physical factors such as boat traffic, biological factors such as introduction of an exotic species, and chemical factors such as pH change. Even if these classes of stressors affect the same trophic levels, the resulting ecosystem disease states have different etiologies because the stress is introduced and propagated by different mechanisms.

This paper presents a toxicological perspective on ecosystem sustainability. I discuss how classical toxicological concepts have to be modified when the experimental unit is an ecosystem. When exposures are high, effects are acute and are often measurable (e.g., fish kill). However, when exposures are low and chronic, effects are often hard to separate from the background. Evidence of high risk for lack of sustainable development is exceedence of ecosystem "threshold criteria."

Key Words
Ecosystem Health
Sustainable development
Ecosystem toxicology
Ecosystem risk
Ecosystem threshold

INTRODUCTION

In Title III of the Clean Air Act Amendments of 1990, the U.S. Congress identifies an initial list of 191 air toxics whose emissions must be regulated because they "...are known to cause or may reasonably be anticipated to cause...**adverse environmental effects...**" As defined by Congress:

> **[T]he term "adverse environmental effects" means any threat of significant adverse effects, which may reasonably be anticipated, to wildlife, aquatic life, or other natural resources including disruption of local ecosystems, impacts on populations of endangered or threatened species, significant degradation of environmental quality over broad areas, or other comparable effects.**

With this definition, Congress has issued a mandate for eliminating toxicological impacts to ensure sustainable ecosystems. As commonly defined, an "ecosystem" is an interacting system of living and non-living components in the environment. A "sustainable ecosystem" is one which is adaptive: it can carry or withstand stresses, is prolonged, and is nourished.

Chemicals affect sustainability by their effects on ecosystem processes. The study of toxicologic responses of ecosystems to contaminants has been termed "ecoepidemiology" by Coulston and Korte (Bro-Rasmussen and Lokke 1984). Ecoepidemiological studies are concerned with describing effects, identifying causes, and determining links and pathways in disease processes affecting populations, communities, and ecosystems. Ecoepidemiological analyses involve ecotoxicological evaluations of many types of test systems which are integrated with other data to provide an assessment of the expected damage to ecosystems. This paper examines parallelisms between classical and ecosystem toxicology.

HOW DO CLASSICAL AND ECOSYSTEM TOXICOLOGY DIFFER?

Classical toxicology uses laboratory studies to examine the effects of chemicals on the individual organisms, organ systems, organs, tissues, or cells used in the test and extrapolate these results to populations and other species, usually humans. Classical toxicology can be viewed from at least three perspectives. One perspective focuses on organ systems and target organs, such as the organs of the circulatory, nervous, and respiratory systems. Another perspective emphasizes effects on physiologic functions such as respiration. A third perspective is endpoints. The left column of Table 1 defines several classical endpoints (NRC 1981). The right column is an initial attempt to identify a corresponding ecotoxicological endpoint; some of these are expanded in Table 2.

Table 1. Classical Endpoints of Toxic Effects

(1) TIME SCALES (Hierarchical order):

Acute toxicity	
The adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 hours.	The definite structural change (e.g., alteration of diversity or loss of species) occurring within a short time after a single or multiple exposures within 24 hours.
Subacute toxicity (14 day)	
The adverse effects resulting from daily doses administered for a period of 14 days. The observation period is the exposure period.	The adverse functional changes (e.g., (decreased productivity, increase or decrease in biomass) resulting from repeated doses over a relatively short period, e.g., arbitrarily, 14 days.)
Subchronic toxicity (90 day)	
Subchronic studies are designed to examine the adverse effects resulting from daily exposure over a portion (<10%) of the average life span of an experimental animal. The observation period is the exposure period plus, e.g., 28 days. These studies provide information on the cumulative toxicity of a substance on target organs and on physiologic and metabolic tolerance of a compound at low-dose prolonged exposure.	The adverse effects to spatial structure and species gradients resulting from repeated exposure for a portion of the lifetime. Many species of plants and animals live decades. Since a redwood lives for 3000 years, a 300 year exposure could be considered "subchronic" by a parallel definition. A functional definition is required: "Subchronic exposure is exposure occurring during <10% of a species normal mating cycle. For insects the exposure might be a few days, for plants 1 or a few months.
Chronic toxicity (> 90 days to years)	
Chronic toxicity tests are long-term studies carried out over a significant portion of the test animal's lifespan. For the rat, chronic toxicity tests are studies longer than 3 months (i.e., >10% of the life span).	Chronic toxicity are the adverse effects resulting from repeated exposures during a significant portion of a species' life. Chronic toxicity is expected to alter structure and function, and result in low numbers of species, possibly low numbers of organisms, and high variance. (For example, addition of deoxygenating wastes to a stream reduces diversity.)

(2) ENDPOINTS (Semihierarchical order):

Mutagenicity	Loss of rare and endangered species. Accelerated development of disease resistance in insects and bacteria.
Teratogenicity (A property of a chemical which causes permanent structural or functional abnormalities during the period of embryonic development.)	Altered succession Selenium-caused collapse of higher trophic species at Kesterson, CA (birds; higher level aquatic species; see Zahn (1986)).
Reproductive toxicity	Lowered reproductive success DDT effect on birds.
Carcinogenicity (A property of a chemical which causes the development of neoplastic lesions during or after exposure to various doses of a test substance by an appropriate route of administration.) (Cancer can be viewed as a loss of, or incorrect operation of cellular feedback mechanisms.)	A property of a chemical which causes feedback mechanisms to operate inappropriately. Expressions of this include decreased population fitness, adverse changes in age-class structure and predator-prey interactions.

(3) TOXICITY, OTHER (Random order):

Acute skin and eye irritation (The skin and eye are barriers between an organism's internal organs and the environment. Acute irritation can be viewed as reduction in the effectiveness of this barrier.)	Reductions in ecotones. An ecotone is a transition between two or more diverse communities. (Decreased barrier protection, such as reduced thickness of layers of dead vegetation protecting soil organisms.)
Allergic sensitization (Acute hypersensitization or allergy is a pathological state resulting from prior sensitization to a specific molecule or structurally related compound.)	Prior exposure reduces the ability of the ecosystem to withstand subsequent exposures. That is, properties such as resilience, resistance, inertia, and elasticity are decreased.
Neurotoxicity (Neurotoxicity is basically a poisoning of the channels involved in information transfer.)	Reduction in system connectivity, i.e., blockages in fluxes. Fire suppression impedes nutrient flow in forests and grasslands.
Hepatotoxicity (Detoxification.)	Capacity of system to store and detoxify.

Ecosystem toxicology is limited by the database available for species of interest. Thus, the Registry of Toxic Effects of Chemical Substances (RTECS) gives data for birds, cat, cattle, horse, chicken, dog, goat, sheep, duck, frog, gerbil, guinea pig, hamster, human, monkey, mouse, pig, pigeon, laboratory quail, rabbit, rat, squirrel, toad, and turkey. Most of the data is for a few species (mouse, rat). Toxicity data for aquatic species are in the database AQUIRE. For the 234 Illinois aquatic species (146 genera) in this database, there is acute data for 895 compounds (Ross et al. 1986). Only 310 are represented by more than 1 species. Sparks (1989) has remarked:

> "Relatively few species of fish have been commonly used as test organisms in laboratory bioassays. The most commonly used freshwater species include fathead minnows (Pimephales promelas), bluegill sunfish (Lepomis macrochirus), channel catfish (Ictalurus punctatus), goldfish (Carassius auratus), common carp (Cyprinus carpio), and rainbow trout (Salmo gairdneri).
>
> "Few toxicity data exists for the majority of Illinois fishes, including those which are likely to be most sensitive, based on what is known of their habitat preferences and historical changes in their distribution patterns. Such groups of fishes include the darters (Family Percidae), redhorse suckers (genus Moxostoma), madtom catfishes (genus Noturus), and some species of minnows (Family cyprinidae). There are 13 endangered and 15 threatened fishes in Illinois whose water quality requirements are largely unknown and will probably not be determined because toxicity testing necessarily involves the death or impairment of hundreds of individual organisms. Of the 199 [fish] species which have occurred in Illinois, toxicity data exist for 84 (Ross et al. 1986), but except for the "standard" bioassay species mentioned above, the data are incomplete, usually consisting of a few chemicals or factors tested under a narrow range of environmental conditions in one laboratory."

It is similarly instructive to examine the universe of phytotoxicity data on terrestrial vascular plants. Fletcher and colleagues (1988) reported that the database PHYTOTOX contained records on 1569 plant species from 682 genera and 147 families.

> "Although this is probably the broadest spectrum of plant taxa ever to be considered in a toxicity database, the numbers of taxa are still quite restricted, inasmuch as the plant kingdom comprises approximately 250000 species, 12000 genera and 300 families....In the database, 42% of the records deal with only 20 plant species, of which 19 are agronomic plants....Approximately 25% of the data deals with only 20 of the 5000 compounds present in the database....In contrast to the abundance of data on the common plant hormones and herbicides, there is negligible or no information on most of the human carcinogens and toxic waste compounds

that are of primary interest to the U.S. Environmental Protection Agency. For example, of the 114 organic chemicals included on the EPA list of priority pollutants, PHYTOTOX contains records for only 40 [including 12 pesticides]."

Classical toxicology is usually defined in terms of an exposure protocol which does not consider the magnitude and duration of the response: "Acute toxicity causes death or extreme physiological disorders to organisms immediately or shortly following exposure to the contaminant. Chronic toxicity involves long-term effects of small does of a contaminant and their cumulative effects over time. These effects may lead to death of the organism or disruption of such vital functions as reproduction" (USEPA 1989, p. 23). In contrast, medical usage recognizes that acute or chronic exposure can have lethal or sublethal effects. Bailliere's medical dictionary (Blood and Studdert 1988) defines "acute" as "having severe signs and a short course of 12-24 h." "Chronic" means "persisting for a long time; the period is undefined and varies with the circumstances. It also has the sense of the disease showing little change or very slow progression over a long period." Ecosystem Health is a medical science so the classification of ecotoxicities must include the exposure duration and the response duration and magnitude. A preliminary classification is attempted in Table 2.

Ecotoxicology studies the effects of chemicals on the structure and function of biotic communities and on their interactions with abiotic components. The "structure of an ecosystem is defined by the abundance and biomass of all populations and their spatial, taxonomic and trophic organization" (Sheehan 1984). Measuring the stability of an ecosystem is therefore a diagnostic goal of Ecosystem Health studies. Attaining and ensuring a sustainable ecosystem requires attaining and ensuring ecosystem stability. Table 3 defines terms used to describe ecosystem stability.

Ecosystem effects of chemicals (NRC 1981; Sheehan 1984) generally are not included in classical toxicology. The outstanding need of ecosystem toxicology is to evaluate subtle, complex effects occurring concurrently in multiple species that are not normally studied by classical toxicologists. The relevant responses, such as changes in species diversity, are difficult to define clearly and unambiguously. Generally, these responses have high measurement variance and unknown accuracy. While many classical responses are components of an ecosystem "health" assessment, they are seldom endpoints per se. System complexity and data scarcity limit the ecotoxicologist's ability to predict the magnitude and significance of toxic effects of chemicals on ecosystems. Models of ecosystem effects have been proposed, but most have substantial data requirements and model results are difficult to interpret. Another limiting factor is that ecosystem toxicology must use limited manipulation and pseudoreplication of the ecosystem in place of the highly controlled and replicated experiments used in classical toxicology.

Table 2. Comparison of Classical with Ecotoxicology

Classical	Ecosystem
ANIMAL RESPONSE	ECOSYSTEM RESPONSE
Acute Exposure, Acute Response	
Example: Strychnine poisoning Exposure to NH_3 or HCl gas	Example: Fish kill due to NH_3 runoff Immediate effects of oil spill e.g., suffocation.
Acute Exposure, Chronic Response	
No standard definition.	The definite structural change occurring over a long time (months to years) after single or multiple exposures within 24 hours.
Example: Organophosphorus-induced delayed neuropathy (OPIDN)	Example: Long-term effects of oil spill
Triorthocresol phosphate (TOCP) in bootleg alcohol caused a massive outbreak of neuropathy in the late 1920's. The paresis of upper and lower extremities was known as ginger jake paralysis.	Loss of critical habitat, delayed toxicity and photo-induced toxicity, bioaccumulation of toxic residues.
Radiation exposure	Radiation exposure (blast)
Loss of organ system function such as red blood cell production and immune system; germ cell effects (mutations).	Loss of species and trophic levels; germ cell effects (mutations, evolutionary "fitness" of species.)
Chronic Exposure, Acute Response	
Copper-molybdenum deficiency in sheep produces an acute syndrome which suddenly occurs after chronic dietary exposure to an excessive copper to molybdenum ratio (>10:1). (The proper ratio is 6:1 and 8-10 ppm of copper in diet.)	Acid rain leaches minerals from thin Adirondack soils thereby permanently changing soil pH. Runoff enters lakes where metals decrease water pH, which in turn increases metal ion solubility. Death ensues from multiple causes.

Chronic Exposure, Chronic Response

Molybdenum toxicosis-copper deficiency	Genetic change due to pollution
Bovine syndrome: Morbidity may approach 80%. Diarrhea with gas bubbles. Emaciation. Decreased milk production. Decreased fertility and lameness. Anemia. Chronically--bone fractures.	a) The classic example is a genotypic color change (from light to dark) in British moths exposed to trees darkened by coal-fire smoke. b) Selenium case in Table 1.
Sheep syndrome: Lambs--ataxia, blindness.	

Idiosyncratic

Abnormal sensitivity, or lack of sensitivity, to exposure by some members of population.	"Most sensitive" species. The species mean LC_{50} for benzene is 5,300 $\mu g/l$ for rainbow trout and 380,000 $\mu g/l$ for _Daphnia magna_. Some _D. magna_ populations had an LC_{50} of 620,000 $\mu g/l$.

TOXIC MODE OF ACTION	ECOTOXIC MODE OF ACTION
Mutagenesis	Genotypic and phenotypic diversity
Teratogenesis	Reproductive effects of individual, species
Carcinogenesis	Fitness (Totter (1981) gives evolutionary basis of carcinogenesis.)
Allergic sensitization	-------

(Parallelism not implied)

SUBSYSTEM (Organ system)	SUBSYSTEM (Trophic Level)
Neuromuscular Hepatic Reproductive	Hierarchial structure altered or destroyed (see Allen et al. 1984).
	Abundance and Biomass
	Reduction in Population Size and Extinction Loss of species with unique functions Species richness
	Community Composition and Species Dominance Species lists Indicator species Biological indices Dominance patterns
	Species Diversity/Similarity
	Spatial Structure
	Stability (see Table 3)

Table 3. Measures of Ecosystem Dynamics and Examples of Their Application (after Holling 1973, Westman (1978), Pimm (1984), Sheehan (1984). Modified from Kerster et al. (1988).)

Characteristic	Definition	Example: Ecosystem subjected to oil spill
Inertia/ Persistence/ Resistance	The degree to which a variable is changed following a perturbation. (Ecological buffering capacity). Units nondimensional and continuous.	Amount of oil that must accumulate over a given area in a given time period to cause a given level of, e.g., local extinction of species X and Y.
Elasticity/ Resiliency	Rapidity of restoration of a stable state following disturbance. Units of time.	Time required to recover initial structure or function following ecosystem damage (e.g. restoration of populations X and Y).
Amplitude	Distance from which the system is able to return to its original state.	Maximum amount of oil that can accumulate in an area such that populations X and Y can be fully restored.
Hysteresis	Degree to which path of restoration is not an exact reversal of the path of degradation.	Degree to which pattern of secondary succession is not an exact reversal of the pattern of retrogression experienced following impact (e.g., were the last species to disappear the first to return?)
Malleability	Degree to which stable state established after disturbance differs from the original steady state	Degree to which new climax ecosystem resembles the initial climax state (e.g., how closely do the species composition and equitability of new climax state resemble old?)
Variance	The variance [or standard deviation (s.d.) or coefficient of variation (c.v.)] of population densities over time. The units are animals squared per unit area (variance), animals per unit area (s.d.), or dimensionless (c.v.).	

Several ecosystem components are "critical" to maintenance of the ecosystem. These characteristics are discussed in a report of the National Academy of Science (1981). Herricks and Schaeffer (1987) identified 8 measurable characteristics likely to be affected by chemicals and gave 44 measures appropriate to individuals, populations, ecosystem biological components, and major abiotic elements (Schaeffer et al. 1988). The 8 critical characteristics (which are not of equal importance in every ecosystem) and classical toxicology analogs are given in Table 4. For example, in the same fashion that respiratory and basal metabolic rates provide system information to the classical toxicologist, photosynthesis and nutrient cycling rates provide system information to the ecotoxicologist.

Table 4 also gives trends that may be expected in ecosystems upon the advent of stress (Odum 1985). Not all of these effects are likely to occur in any particular, newly stressed ecosystem. The intensity and duration of the stress are both important in this respect. For example, stress in many freshwater aquatic ecosystems will cause large changes in species composition (such as in benthos and phytoplankton) before changes take place in ecological functions such as respiration or primary production. "In such a case the toxic threshold for sensitive taxa occurs at a lower intensity of stress than does the toxic threshold for function, which may be carried out at a community level irrespective of the species composition. Therefore, changes in species composition and demographics would be expected to be relatively sensitive indicators of the initial ecosystem response to the advent of a low or moderate intensity of stress" (Freedman 1989, p. 320). However, in many terrestrial ecosystems, such as forests (Schindler 1988) and grasslands (Schaeffer 1989), the reverse may be found. "[A] moderate intensity of stress may affect functional attributes such as photosynthesis, respiration, and nutrient cycling well before there is mortality of individual plants or elimination of species" (Freedman 1989, p. 320).

Table 4. Classical Toxicological Analogs of Ecosystem Critical Characteristics

Ecosystem "Critical" Characteristic and trends in stressed ecosystems[1]	Classical Toxicological Analog
I. Habitat is suitable for maintaining diversity and reproduction of organisms at evolutionarily acceptable levels. *Stressed ecosystem trends include:* *Ecosystem becomes more open.* *Successional trends reverse.* *Parasitism and other negative interactions increase, mutualism and other positive interactions decrease.* *Sensitive genotypes are replaced by more tolerant genotypes.*	Habitat is suitable for maintenance of test individuals.
II. Phenotypic and genotypic diversity exists and is maintained among organisms. *Stressed ecosystem trends include:* *Large species lost.* *Decrease in lifespans of organisms or parts (e.g., leaves).* *Species diversity decreases and dominance increases.*	Usually, phenotypic and genotypic similarity of test organisms is desired and maintained.
III. A robust food chain supporting the desired biota exists. *Stressed ecosystem trends include:* *Proportion of r-strategists increases.* *Food chains shorten.*	Metabolism.
IV. An adequate nutrient pool for desired organisms exists. *Stressed ecosystem trends include:* *Nutrient loss increases (i.e., system becomes more "leaky").*	Metabolic reserves.
V. Nutrient cycling is adequate to perpetuate the ecosystem. *Stressed ecosystem trends include:* *Food chains shorten.* *Maintenance to biomass structure (P/B and R/B) ratios increase.* *Nutrient turnover time increases.* *Horizontal transport increases and vertical cycling of nutrients decreases.*	Catabolism.

VI. Energy flux is adequate to maintain the trophic structure. *Stressed ecosystem trends include:* *Ecosystem becomes more open and internal cycling is reduced.* *Efficiency of resource use decreases.* *Importance of auxiliary energy increases.* *Community respiration decreases.* *Production/respiration becomes unbalanced (i.e., P/R < > 1).*	Respiration, metabolism, catabolism, are adequate to maintain normal growth rate.
VII. Feedback mechanisms for damping undesirable oscillations are in place and adequate. *Stressed ecosystem trends include:* *Redundancy of parallel processes declines.* *Successional trends reverse to to earlier stages.*	Feedback mechanisms, such as hormone levels, are in place and adequate.
VIII. An adequate capacity to temper toxic effects, including the capacity to decompose, transfer, chelate or bind anthropogenic inputs to a degree that they are no longer toxic within the system. *Stressed ecosystem trends include:* *Efficiency of resource use drops.* *Ecosystem becomes more open so that inputs and outputs become more important as internal cycling is reduced.* *Size of organisms decreases.* *Species diversity decreases.*	Detoxification mechanisms, including metabolism, binding and excretion have adequate capacity and rate.

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[1] Ecosystem trends adapted by author from Odum (1985).

THE EMERGING SCIENCE OF ECOSYSTEM HEALTH

The bases of the new science of Ecosystem Health are: determination of the existence of stress; systematic study of the effects of stress on ecosystem structure and function; and relief of stress to allow normal ecosystem functioning. The science of Ecosystem Health is analogous in its goals and methods to those of the human and animal health sciences (Table 5). Evolution of the science of Ecosystem Health will lead to development and use of standardized diagnostic methods as "markers" of effect, specialized language, catalog of diseases, ecosystem specialists, and in the fullness of time, treatment methodologies for ill ecosystems. The conceptual stage for development of the science of Ecosystem Health has been set thus (Schaeffer et al. 1988).

> "The classical definition of an ecosystem couples interacting living organisms and nonliving components of the environment to form one physical system and grew from the recognition that definable and describable units existed in nature. Today we approach the protection, management, or restoration of natural environmental conditions using conditions which may or may not be ecologically relevant. Although the maintenance of ecosystem integrity requires more than single species management or protection, environmental protection is constrained by analyses which incompletely integrate ecosystem complexity and provide results with only limited ecological relevance. It is difficult to extrapolate laboratory testing to actual ecosystem effect.... [M]easures of human or nonhuman animal health, and the clinical analysis of factors which contribute to a definition of a state of health, provide useful analogs to the problems faced by environmental managers attempting to maintain the integrity of ecosystems."

> "Environmental decision making is limited, and is often fundamentally flawed, because of the inability to relate data in an ecosystem context. The issue is not always that insufficient or inadequate data are available....[but] that scientists and engineers fail to extract all the information possible from existing data sets. The lack of data may be the excuse for the absence of decisions....[A] more pertinent issue is the lack of a common set of analysis approaches....which translate data on the complexity of ecosystems into simple and understandable information about state or condition which will support decision making."

Table 5. The Tools/Concerns of Classical and Ecosystem Physicians[1]

HUMAN/VETERINARY PHYSICIAN	ECOLOGICAL SYSTEM PHYSICIAN
Compendium of diseases is available.	Diseases of ecological systems largely undefined.
	No terminology to describe "ecosystem health status" and "diseases".
Wide body of reference data available for "standard man".	Body of data for ecological systems contains some information on normal patterns of change and variability in ecosystems.
	Virtually no data from long-term, whole ecosystem monitoring.
	A "standard ecological system" cannot be defined at this time.
Effect thresholds (e.g., respiration and heart rates, body temperature) are well quantified.	Few or no qualitative or quantitative effect thresholds.
Many types of diagnostic tools available.	Virtually no proven diagnostic tools.
The ranges of use of diagnostic tools and the interpretation of data are well defined.	Interpretation of data in many cases is not practicable or possible.
Concerns of toxicologist:	Concerns of ecosystem toxicologist:
(1) Effects of poisons on organisms considered as individuals.	(1) Effects of poisons on structure and function of ecological systems.
(2) Time to signs, survival time, proportion of surviving individuals, and relations among these and dose of toxin are used for effect assessment.	(2) Identification of alternative futures of stressed ecological systems and factors affecting probability that a particular future will be realized.
(3) Design of therapy for affected individuals.	(3) Design of therapy for affected ecosystems.

[1] Adapted from Schaeffer et al. (1988).

MARKERS OF EFFECT: ECOSYSTEM THRESHOLD CRITERIA

The issue of sustainability can be viewed as the maintenance of ecosystem stability. An ecosystem as stable if, and only if, the variables all return to the initial equilibrium following their being perturbed from it (Pimm 1984). An ecosystem is locally stable if this return is known to apply only certainly for small perturbations and globally stable if the system returns from all possible perturbations. Based on classical toxicology we infer that instability occurs when an ecosystem "threshold" is exceeded. Thus, Woodwell (1975) asked:

> Is it reasonable to assume that thresholds for effects of disturbance exist in natural ecosystems? Or are all disturbances effective, cumulative, and detrimental to the normal functioning of natural ecosystems?
>
> The question is analogous to the classical question of hazards of ionizing radiation and other toxins. If small exposures in addition to background have no discernible effect, but larger exposures do, then it is common to speak of the maximum exposure that has no effect as a "threshold."

The existence of ecosystem thresholds, real or apparent, are assumed (de facto) to provide a practical basis for regulation of a pollutant or other types of stressors, such as vehicle traffic. However, it may be that exact criteria cannot be developed. Thus, based on whole ecosystem radiation studies, Woodwell (1975) concluded that:

> "If we seek thresholds along [an exposure] gradient, we can find them, but not at the ecosystem level. The thresholds are for survival of species, for the elimination of trees, for the invasion of adventives, for measurable effects on growth. If we measure effects on growth, we find that greater refinement in analysis shows effects at lower concentrations. Any threshold is arbitrary."
>
> "...[I]f thresholds exist for effects of disturbance, they are few, and they lie at exposure levels below those at which they can be resolved by most current studies of ecosystems per se."

A marker of effect is evidence that an (apparent or real) ecosystem threshold criterion has been exceeded. A preliminary definition of a what Schaeffer and Cox (1990) term a "functional" ecosystem threshold criterion is:

> **"Any condition (internal or external to the system) which, when exceeded, increases the adverse risk to maintenance of the ecological system."**

Systematic development of functional ecosystem threshold criteria is a significant new research area which encompasses and integrates the judicial, political, social, and economic disciplines, and the biological, chemical, and physical sciences.

Scientific criteria are conveniently classified into three categories. Biological threshold criteria encompass a wide range of species or community responses. For example, Karr's (1981) Index of Biotic Integrity is computed using 11 characteristics of the midwest fish community. Threshold criteria can be developed using the physical properties of the abiotic components of the environment. For example, the Predicted Index of Biotic Integrity (PIBI) (Hite and Bertrand 1989) uses stream physical characteristics to predict the maximum possible IBI score. Criteria may also be based on the chemical properties of the abiotic components. Changes in energy flux relate the abiotic to the biotic components, and could be a basis for threshold criteria. Scientific criteria can be developed through the formal process of hypothesis testing (e.g., IBI), modeling (e.g., PIBI), or consensus (e.g., Delphi) procedures (Brown et al. 1970).

Development of scientific criteria is a complex undertaking which involves, initially, identification of appropriate biotic measures and, subsequently, controlled testing and refinement. A comprehensive effort will require identification of existing criteria, measures which could be readily modified to provide initial criteria, and a process/data requirements for developing criteria. Each type of ecosystem has unique species, abiotic components, and interactions which must be dealt with by experts in the ecology of that type of ecosystem. For example, while a threshold for "sufficient nutrient cycling" is applicable to all types of ecosystems, the criterion value and method for its measurement can be different for aquatic, prairie, and forest ecological systems.

RISK ASSESSMENT FOR ECOSYSTEMS

Most risk methods for chemicals focus on single chemicals and human cancer. Comprehensive methods are not available for assessing the toxicity/risks for mixtures, time-ranging concentrations (however, see Mancini 1983) and ecosystems. A natural species may be simultaneously subjected to several stressors. In a population subjected to substantial pressure, the stability will be low and the individual risk factors can interact synergistically to produce a total risk which is much greater than the simple sum of the individual risks. Barnthouse and colleagues (1990) demonstrated this for fisheries populations using elegant, powerful, risk models that combine laboratory toxicity test data with life history data. For example, models showed that because of differences in life history, the heavily exploited species (Chesapeake Bay striped bass) had a much lower capacity to sustain contaminant-induced mortality (from trifluralin) than did Gulf menhaden. Their results suggest that consideration of life history may be important for site-specific assessments, but that the substantial differences in uncertainty associated with different types of test data are of much greater concern for screening-level assessments.

Species show varying sensitivities and responses to toxic substances, so it is generally not possible to deduce the response of an ecosystem from the response of a single species. Yet, the predictive relationships which have been developed among many species suggest that relationships can be developed which use the response of a single species (or a small number of species) to predict the response of an ecosystem. According to published criteria, a species has ecological importance if it: 1) provides more than 20% of total biomass at a given trophic level; 2) provides a primary food supply (more than 30%) to an organism with economic or social value; 3) provides a secondary food supply (> 20%) to an organism with economic or social value; 4) is a primary producer or primary producing group that provides more than 20% of the food base for primary consumption; 5) is a dominant predator than consumes more than 15% of a given species; or 6) provides important habitat space for other organisms.

Because of uncertainties in environmental monitoring (exposure) and biological (response) data, susceptibilities of individuals and species (Storer 1975; Totter and Weinberg 1977), and the like, the exposure and the dose-response curves, and hence the risk curve, cannot be accurately determined (supra Barnthouse et al. (1990)). However, if uncertainties are small enough to permit at least confidence region estimates for the component curves, then probability convolution methods (Kaplan 1981; Springer 1979), can be used to compute simultaneous confidence regions for the risk frequency curve (Clarke and Schaeffer 1990). Point estimates for the various risk statistics of interest are thus replaced by approximate numerical confidence interval estimates for these statistics.

CONCLUSION

This paper has attempted an initial comparison between those aspects of classical toxicology and the emerging field of ecosystem toxicology related to the development, characterization, and maintenance of sustainable ecosystems. Although many components of ecosystem toxicology can be analogized to elements of classical toxicology, the ecosystem toxicologist must guard against oversimplification. There are at least eight differences which must be emphasized.

(1) The multispecies structure of an ecosystem cannot be fully analogized to an individual or a laboratory colony.

(2) Genotoxic effects in a population affect a species' evolutionary fitness which is not analogous to survival of an individual.

(3) Energy and nutrient flows and balances in an ecosystem include the abiotic components and the biotic components at numerous trophic levels, and these are not always

identifiable or measurable.

(4) Environmental factors can cause large geographic and annual fluctuations (coefficient of variation > 30%) in population measures such as numbers of organisms, age class distribution and diversity. Classical toxicologists accustomed to relatively low variability in inbred animals and statistical criteria of $\alpha = 0.05$, power = 0.8 to judge treatment differences, will have to adjust to the use of more liberal criteria, such as $\alpha = 0.1$ or $\alpha = 0.2$, power = 0.6., for judging ecosystem differences.

(5) The ecotoxicologist works in complex systems which can have multiple mechanisms for tempering toxic effects. Consequently, an effect which is "significant" to the classical toxicologist may not be found by the ecotoxicologist. For example, because nutrient recycling was very rapid in a prairie ecosystem exposed to both chemicals and heavy vehicle traffic, a field study did not find exposure effects although significant mortality was found in plants exposed in the laboratory to these chemicals (Schaeffer et al. 1990).

(6) Classical toxicology relies on replication whereas ecosystem toxicology is limited, at best, to pseudoreplication (Hurlbert 1984) through use of devices such as limnocorals.

(7) The concept of an "ecosystem threshold criterion" is very new, and until the theoretical concepts needed to develop criteria and measure effect are identified, ecosystem toxicology will continue to emphasize studies of acute effects resulting from high-level acute exposures and not studies of effects resulting from low-level chronic exposures.

(8) The complexity of the ecosystem must never be underestimated or over-simplified. For example, small intermittent streams are often expendable in the human system of values. However, these are important to the sustainability of larger perennial streams because they carry a diverse, annually highly variable, biota which contribute both to an individual species' gene pool and to a community's species pool.

Ecotoxicology is concerned with the assessment of ecosystem risk and with development of methods which will enable resource managers to minimize ecosystem risk. We can (intuitively or quantitatively) express "risk" to an ecosystem as a metric between 0 and 1. If ecosystem risk is low, then ecosystem health is high and, by definition, the ecosystem is sustainable. Being ecosystem risk assessors, ecotoxicologists will therefore play a key role in any effort to maximize ecosystem sustainability.

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SUSTAINABILITY OF FISH POPULATIONS: IMPLICATIONS FOR TOXICOLOGY. L. W. Barnthouse, Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831-6036 (615-574-7393).

Sustainable development implies development of human societies in a way that maintains populations and ecosystems in desirable states. For fish populations, sustainability implies maintenance of physical habitat, water quality, community structure, and population structure. Management for sustainability requires that we (1) define the essential characteristics of the systems we wish to preserve, and (2) determine how these characteristics respond to multiple environmental stresses with complex distributions in time and space. Independent management of single stresses (fishing, point-source effluents, nonpoint-source inputs) must be replaced by integrated management of all stresses. The past role of toxicology in environmental management has been limited to determining lethal or sublethal exposure levels for single toxic chemicals or effluent mixtures. Management for sustainability of populations will require integration of toxicological methods with management tools derived from fisheries science and ecology. This paper reviews progress that has recently been made in this direction and discusses changes in testing strategies required to fully integrate ecotoxicology into sustainable management schemes.

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Monitoring for Sustainable Development

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Extended Summary

One of the key concerns regarding sustainable development is developing and implementing monitoring strategies to measure the state of ecosystems, resources, and conditions sustaining human health. Monitoring for sustainable development requires measurement of system attributes. This ecosystem approach involves first establishing monitoring goals consistent with sustainable development, then developing a sampling strategy to meet the monitoring goal.

Most monitoring programs measure only "simple" variables whose behaviour is determined by only a few processes. Examples would be the concentration of phosphorus in water or the concentration of dioxins and furans in fish tissues. Monitoring for sustainable development must measure also "complex" system attributes with multiple functional linkages to many other ecosystem variables. Such attributes include trophic structure, nutrient flow, productivity of trophic levels and of ecosystems, energy flow, ecosystem state, and resilience. Ecosystem state is a description of a characteristic qualitative functioning mode of an ecosystem. Major functional pathways, key and dominant species, and general mode and rate of production remain relatively constant. Change to different ecosystem states implies a qualitative or major quantitative jump to different ecosystem characteristics. Resilience is the ability of a system to oscillate but remain in the same qualitative state (ecosystem state in this discussion). These system attributes are both more abstract and more complex. In many cases, effective measures or indicators of these attributes have yet to be developed. For example, although total phosphorus at spring overturn has been found to be a useful indicator of trophic state for lakes that stratify, we do not have a similarly predictive measure of trophic state for flowing or marine waters. Useful indicators or measures of resilience are still to be developed as well.

Monitoring goals for sustainable development focus on gathering information that will anticipate changes in ecosystem state, a difficult goal considering our lack of understanding of the alternate states in which an ecosystem might exist. An

ecosystem might change from its current state to a different, yet sustainable, ecosystem. One important goal of monitoring for sustainable development is to estimate the probability and risk of such a state change.

In this perspective the monitoring program should, at each "sampling," provide risk status. Implementing monitoring for risk status will require measuring ecosystem state, ecosystem resilience, and probability of a state change. Measurement and development of indicators of ecosystem state, resilience, and probability of a state change requires an extensive and intensive development effort.

In addition to knowing the risk status it is necessary to evaluate the consequences of state changes. Predicting the consequences of state changes involves evaluation of alternate states. Managers must be able to evaluate the social/environmental desirability of potential alternate states versus the current state of the ecosystem. Evaluation of alternate states is difficult and always entails uncertainty. Some methods, such as modeling, microcosm and field experimentation, and case studies of managed ecosystems, are available as starting points to predict alternate states. In some cases, alternate states could have greater overall value, including both monetary and non-monetary considerations, to humans. This evaluation is an opportunity for application of decision support systems and public input processes.

WATER QUALITY GUIDELINES AND OBJECTIVES FOR SUSTAINABLE DEVELOPMENT

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ABSTRACT

To realize the goals of sustainable development and bring environmental factors into the mainstream of decision making, we must develop the scientific, environmental framework necessary to move sustainable development from a vaguely defined political philosophy to a practical, operational concept. Water quality guidelines and objectives define realistic and scientifically defensible measures and goals of environmental sustainability that can be incorporated into the decision making process alongside conventional economic and social criteria. Water quality guidelines and objectives are now widely used across Canada. The "goals and yardstick" value has potentially broad applications to the sustainable development of water resources; i.e. environmental impact assessment, site rehabilitation, state of the environment reporting, and the development of standards for aquatic ecosystem protection. With reference to Canadian programs, we discuss current trends in the development and application of water quality objectives to sustaining aquatic ecosystems. Topics include the development of water quality objectives for transboundary water management, the development of ecosystem objectives for Lake Ontario and the importance of public participation, and the move from traditional "end-of-pipe" approaches to environmental protection and regulation to approaches incorporating specific water quality and ecosystem objectives for receiving waters.

INTRODUCTION

Sustainable Development

Since the World Commission on Environment and Development (Brundtland Commission) released its landmark report in 1987, the concept of sustainable development has pervaded social, economic and ecological theory in Canada and set a new course for the way we view the environment and our relationship to it. Sustainable development, perhaps more accurately referred to as "environmentally sustainable economic development", has been variously described as "*development which meets the needs of the present without compromising the ability of future generations to meet their own needs*" (World Commission on Environment and Development) and "*development which ensures that the utilization of resources and the environment today does not damage prospects for their use by future generations*" (National Task Force on Environment and Economy). Though definitions may vary, they are united by a common ethic - the right of future generations to the resources of the planet - and are based on the premise that we can have economic development over the long-run only if we maintain long-term health and integrity of the environment.

The Challenge of Sustainability - From Rhetoric to Reality

"... the beguiling simplicity of the term sustainability has placed it in grave danger of becoming so totally accepted politically, that it will become meaningless; languishing as a "good idea" which cannot be put into practice ..." (O'Riordan, 1988)

Though the principles of sustainability may be clear, precise definitions and measures of sustainability remain elusive (e.g. Shearman, 1990). The current challenge is to move from a vaguely defined political philosophy to an operational, practical concept or strategy (Slater, 1988). We must provide credible goals and measures of environmental sustainability so that environmental factors can be shifted into the mainstream of decision-making at all levels as the basis for development of environmentally sustainable policies, programs and projects (Environment Canada, 1990).

Water Quality Guidelines and Objectives

Water quality guidelines are an important component in developing an operational framework for sustainable development. They provide scientifically defensible, precisely defined numerical limits or narrative statements for sustaining aquatic environmental quality and uses that can be incorporated into the decision-making process alongside conventional economic and social criteria. Water quality guidelines are based on an integration of environment/economy data, such as environmental response to potential human-induced stresses (e.g. eco-toxicology) and water uses; uniting science, technology and public values (Hamner, 1989) to define realistic and scientifically defensible goals and measures for sustainability.

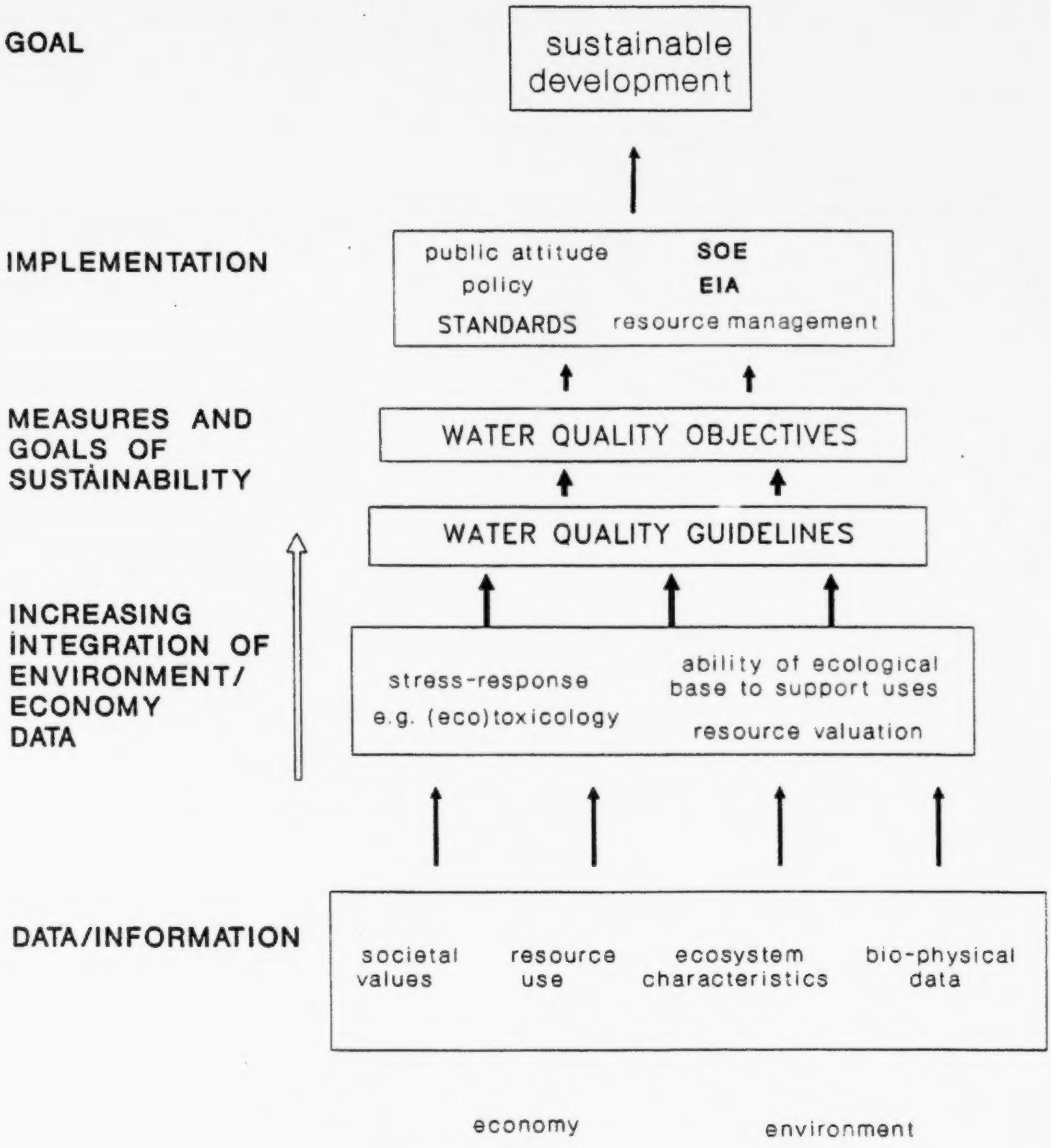
The "goals and yardstick" function has potentially broad application to such areas as impact assessment, state of the environment reporting and environmental management and rehabilitation. **Water quality guidelines** can be used directly to assess water quality issues and concerns but they also serve as the technical basis for the development of **water quality objectives** - numerical concentrations or narrative statement established to support and protect the designated uses of water at a specified site (CCREM 1987b). Water quality objectives, in turn, provide a framework for the development of **water quality standards** - objectives that are recognized in enforceable environmental control laws of a level of government. Development and harmonization of standards and legislation for environmental protection has been identified as a primary goal for sustainable development in Canada (CCREM, 1987a). Figure 1 indicates the theoretical placement of water quality guidelines, objectives and standards in E.W. Manning's "building blocks" of sustainable development (Environment Canada, 1990).

The nature and role of water quality guidelines and objectives in the sustainable development of Canada's water resources will be discussed with reference to several Canadian examples.

CANADIAN WATER QUALITY GUIDELINES

Canadian provinces began to develop water quality objectives in the 1960s and 70s. In 1987, the CCME (formerly CCREM) published the Canadian Water Quality Guidelines to provide a nationally consistent scientific basis for developing water quality objectives. The Canadian Water Quality Guidelines synthesize a broad base of ecotoxicological, environmental and human use information to define

FIGURE 1: BUILDING BLOCKS FOR SUSTAINABLE DEVELOPMENT


GOAL
sustainable development
IMPLEMENTATION
public attitude
policy
STANDARDS
SOE
EIA
resource management
MEASURES AND GOALS OF SUSTAINABILITY
WATER QUALITY OBJECTIVES
WATER QUALITY GUIDELINES
INCREASING INTEGRATION OF ENVIRONMENT/ ECONOMY DATA
stress-response
e.g. (eco)toxicology
ability of ecological base to support uses
resource valuation
DATA/INFORMATION
societal values
resource use
ecosystem characteristics
bio-physical data
economy
environment

adapted from Environment Canada (1990)

benchmark values based on the degree to which aquatic systems can withstand or sustain contamination or other perturbations without impairment of important uses or biotic integrity (aquatic life). Guidelines specify water quality (determined by the kinds and amounts of matter which is dissolved and suspended in water such as **bacteria, pesticides and metals**) for single uses such as livestock watering and recreation. Specifying parameters for distinct uses (including aquatic life), maintains maximum flexibility in application of the guidelines to development of site-specific **objectives** which define the water quality necessary to sustain all existing and intended uses at a particular site. This guidelines/objectives approach provides a clear strategy for dealing with the multi-functional nature of water resources and provides realistic, integrated measures or goals for sustaining aquatic resources that can be tailored to specific uses, societal values, and environmental characteristics of a site.

Canadian Water Quality Guidelines (CCREM 1987b) are now widely used across Canada and address a range of potential uses (recreation, irrigation, livestock watering, municipal, freshwater aquatic life). The guidelines contain recommendations for chemical, physical, radiological and biological parameters necessary to protect and enhance these uses, including the forms and fate of the parameters. For example, more than 50 parameters have been addressed in the formulation of guidelines for freshwater aquatic life.

EMERGING TRENDS IN DEVELOPMENT AND APPLICATION OF WATER QUALITY OBJECTIVES

Water Quality Objectives for Transboundary Water Management

Water quality objectives have played a key role in transboundary water management in Canada. Objectives were set for the Red River in 1969, the Great Lakes in 1972 and 1978, and the Poplar River in 1981. Since 1969, the Prairie Provinces Water Board (a federal-provincial board which coordinates interprovincial water management for the prairie provinces) has been working to develop water quality objectives for effective interprovincial water quality management. In 1973, the PPWB Water Quality Objectives were adopted as a first step in the process of establishing an interprovincial water quality management strategy. These objectives were defined as water quality "aims or goals" toward which to strive in order to ensure uses and quality are maintained (sustained) in light of existing and anticipated stresses on the system (e.g. hydro-electric development) (PPWB,1989).

The PPWB incorporates water quality objectives as a cornerstone in delivering its water quality mandate (PPWB, 1990) which "encourages the protection, restoration, and enhancement of waters and related ecosystems for the benefit of present and future generations" (i.e. sustainable development). The PPWB Water Quality Agreement states that "in recognition of the need to use water quality objectives to maintain or enhance the quality of water and to avoid or resolve conflicts, the Board will apply water quality objectives to agreed upon interjurisdictional reaches."

PPWB water quality objectives encompass a broad range of uses that promote an integrated, ecosystem approach to water management. Uses considered in the formulation of objectives include domestic, aquatic life, wildlife, irrigation, industry, livestock, and recreation. Site-specific objectives are based on a consideration of all existing and potential uses, parameters of concern for protection of these uses, and identification of parameters that must be limited to protect uses. Ultimately, site-specific objectives are developed which define maximum acceptable levels that should not be exceeded for each parameter, to protect **the most sensitive use.**

The PPWB example clearly illustrates the role and importance of water quality objectives in providing precise and scientifically defensible measures of sustainability that can be incorporated into the decision-making process. Importantly, they serve as the basis for a "regulatory" framework to protect and sustain water resources based on a broad, multi-functional perspective which integrates environmental and societal values.

Ecosystem Objectives for Lake Ontario

Over the past decade, our understanding of "water quality" has expanded to encompass the concept of aquatic "ecosystem health". Resources are part of a complex and inter-linked system and long-term sustainability of our water resources and the many related uses and benefits depends on a holistic approach based on the protection of ecosystem integrity (Nelson and Eidsvik, 1990). Specific chemical objectives, though a critical component of a coherent water management framework, cannot be pursued out of context of the larger interactive system and water quality objectives must ultimately be set on the basis of long-term ecosystem needs (Rivers and Williams, 1989).

The development of ecosystem objectives for Lake Ontario is a prime example of the expanding nature and role of water quality objectives. The program involves several hierarchical components; a statement of **goals** based on societal values; ecosystem **objectives** for various ecosystem components required to meet the goals; and the development of quantitative **indicators** to measure progress toward each objective (Ecosystem Objectives Working Group, 1990).

Three goals for Lake Ontario have been identified as the basis for sustaining ecosystem integrity and uses on the long-term; 1) self-producing diverse biological communities; 2) levels of contaminants that do not limit the use of fish, wildlife and waters by humans or cause adverse health effects in plants and animals and ; 3) recognition of society's impact on the ecosystem and responsible stewardship. To achieve these goals, five ecosystem objectives have been identified; 1) diverse, self-sustaining aquatic communities; 2) a diverse, self-sustaining wildlife community in the basin; 3) water, plants and animals free from contaminants at levels affecting human health or aesthetics; 4) sufficient quality and quantity of offshore, near-shore, wetland and upland habitat to support wildlife and ; 5) responsible stewardship.

The final step in the process is to develop the scientific measures (indicators) by which progress towards the broad ecosystem objectives can be evaluated.

Public Consultation and Education

" If the right environmental objectives are in place and the opportunity exists for society to respond in a rationale fashion, then sustainable development with respect to water resources is attainable" (Rivers and Williams, 1989)

No matter how scientifically sound or laudable our goals for environmental sustainability may be, they will be ineffective if society does not participate fully in their development and delivery. Nelson and Eidsvik (1990) emphasize that an earlier shift towards an "eco-development" model was only partially successful because development and conservation were still basically envisioned as separate worlds. Sustainable development, by definition, serves to conceptually link these two worlds and the public must be involved if the process is to work.

Traditional water quality guidelines and objectives, by their nature, incorporate societal values and are communicated in terms that are readily understood and accepted by the public. In essence, water quality objectives may be seen as the "flesh on the bones" of the larger societal goals such as "fishable, swimmable water" (Hair, 1989). As water quality issues expand in scope and complexity to embrace ecosystem concepts, the public must play an increasingly important role in the development of water quality objectives to ensure that new and emerging goals reflect societal values and are understood and supported by the public.

Public participation is a cornerstone in the development of ecosystem objectives for Lake Ontario and has been incorporated fully into the process as mandated in the Great Lakes Water Quality Agreement. Part of the process was to identify the mechanisms by which the public input could be maximized. Several possible approaches were advocated including a public advisory committee, public workshops and hearings, public representation on work groups, public representation on committees, public notification with review and comments, correspondents and communication materials (Binational Objectives Development Committee, 1989). The consultation process is ongoing and a combination of these approaches has and will be used. For example, Great Lakes United has spear-headed public meetings throughout the Great Lakes region and the public has been invited to participate on work groups.

As water quality goals evolve to reflect a new understanding about the environment and our relationship to it, public education becomes an increasingly important part of the consultation process. Society must understand the stresses on the environment and the role and nature of objectives, if they are expected to participate effectively in the consultation process and to act in the ways necessary to achieve the objectives for a sustainable environment. Education and communication are an important component of the Great Lakes Program. If momentum towards the goal of sustainable development is to be maintained in light of increasing complexity in issues and actions, then both consultation and education must be considered as integral components of the process for development of water quality/ecosystem objectives.

Beyond the "End-of-the-Pipe": Water Quality Objectives in Environmental Management and Regulation

There is a growing recognition that the traditional "end-of-the-pipe" approach to water quality protection falls short of the goal to protect and sustain aquatic ecosystems (e.g. Rivers and Williams, 1989). Effluent discharge standards that specify contaminant concentrations do not take into account the total mass loadings to a water body that may ultimately exceed the assimilative capacity of the ecosystem. Neither can end-of-the-pipe standards account for the cumulative impact of several toxins from both point and non-point sources. There is a need for a more environmentally responsive approach to the regulation of industrial water pollution; one that incorporates water quality objectives for receiving waters.

Sprague (1990) describes a three-pronged regulation strategy for industrial water pollution that maximizes protection and maintenance of aquatic ecosystem integrity. His approach incorporates three tactics: 1) end-of-pipe (numerical limits for specified variables and toxicity); 2) site-specific (numerical limits for specified variables and sublethal toxicity at the edge of the mixing zone) and 3) ecological survey to assess effectiveness (changes in specific community variables). End-of-pipe limits provide a measure of control at the source but do not deal with important ecological questions and may not achieve environmental protection because characteristics of the receiving water body are not considered. Site-specific limits, which are analogous to the traditional guidelines/objectives values described earlier, overcome limitations of the first tactic and eliminate or prevent ecosystem impairment beyond the edge of the mixing zone. Sprague emphasizes that tactic two should be the strictest part of the enforcement package. Finally, ecological survey is recommended as a quality control check to ensure that the objectives applied at the edge of the mixing zone and at the end-of-pipe are effective in sustaining the aquatic community. This final tactic requires that we develop numerical values for "meaningful change" in communities (e.g. diversity, abundance) and whole organisms (physiological/biochemical) as indicators of ecosystem health. The need for such biological measures in addition to traditional chemical and physical measures of water quality, as part of a comprehensive water management strategy is becoming increasingly apparent as water quality goals broaden to encompass ecosystem protection and sustainability (e.g. EPA, 1990).

In Canada, pollution regulation and legislation is still largely entrenched in the first tactic (i.e. effluent standards). However, Canadian Water Quality Guidelines provide the basis for implementation of tactic two. The Great Lakes ecosystem work serves as a model for providing credible goals and measures for tactic three. If we are to deliver on the promise of sustainability, then more regulatory emphasis must be placed on tactics two and three (Sprague, 1990) .

CONCLUSIONS

Water quality guidelines and objectives provide an important framework for sustainable development of water resources, defining clear and unequivocable goals and measures of sustainability that can be incorporated into the decision-making process . Though they are now extensively used across Canada, there is a need to incorporate water quality objectives more fully into the regulatory framework.

Emerging trends in the development of water quality objectives have followed the same general trajectory as our understanding of the environment and our relationship to it - shifting from an anthropocentric perspective ("drinkable, swimmable water") to an ecosystemic view that recognizes the need for ecosystem level goals and measures.

Ecotoxicology is a critical component of water quality guideline and objective development but the research base must be expanded at the community and ecosystem level. The development of biological measures of "water quality" and biological monitoring tools as a compliment to the traditional chemical objectives and measures is becoming an increasingly important component of a coherent framework for aquatic ecosystem protection.

There are important opportunities to build on the model provided by water quality guidelines and objectives in sustainable management of resources. Development of "environmental quality" guidelines that incorporate other media such as sediments and soils, and other resources such as wetlands and coastal waters, will provide the framework for a more comprehensive approach to sustainable development.

Finally, public participation is critical to ensuring that emerging ecosystem goals reflect long-term societal values and have the support and understanding of the public. It is a well accepted maxim that "where the people lead - the leaders will follow".

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PLATFORM SESSION

Genotoxic/Cytotoxic Bioassays

Chair: C. Metcalfe

EVENT-FREQUENCY RELATIONSHIP BETWEEN TWO GENOTOXIC END-POINTS AND CELL DEATH IN CULTURED FISH CELLS

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Abstract

Genotoxins are physical or chemical agents capable of damaging the genetic components of cells (chromosomes and DNA) at concentrations below those which are required to produce classic toxic end-points. The lesions produced by genotoxic agents are broadly divided into two major types; (1) macrolesions, which are visible with the aid of a microscope, and (2) microlesions, which are identifiable only by indirect means. Because the chromosomes of most fish species are extremely small, most standard mammalian cytogenetic techniques do not work well due to poor resolution. Consequently, the anaphase aberration test (aat) was adapted as a genotoxic test for fish. The lesions visualized by this procedure are large and easily quantified, but result from a variety of different types of damage. In order to clarify the relationship between anaphase aberrations (aa) and other types of toxic and genotoxic responses, we used a known genotoxin, MNNG, as our model and compared anaphase macrolesions with a standard end-point (cell death) and a microlesion (foreword mutation).

BF-2 cells derived from the bluegill sunfish were exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and examined for cell death, aa, and the induction of forward mutations. MNNG produced significant increases in all three parameters relative to control cells. Cells exposed to MNNG concentrations of greater than 0.1 µg/ml for 24 hr showed a significant decrease in viability, but >10 µg/ml were required to elicit 90-100% mortality. The frequency of aa peaked at 20-25% abnormal anaphase cells above 2 ug/ml. When these same cells were examined for ouabain resistant mutants (oua^r), the mutation frequency increased linearly from a background rate of 1×10^{-7} mutants/10^6 clonable cells to greater than 1500 mutants/10^6 clonable cells. To gain insight into the mechanistic relationship between these end-points, the dose responses from the three assays were compared with each other. There was a linear relationship between aa and cell death (R^2= 0.95). The relationships between oua^r and anaphase aberrations and cytotoxicity are best described by exponential (R^2= 0.92) and polynomial (R^2= 0.98) equations, respectively. These findings provide indirect evidence that aa and cell death may be mechanistically related, while mutagenesis is mechanistically distinct from both aa and cytotoxicity. Consequently, the genotoxic response should be considered a distinct end-point, equivalent to any other standard toxic end-point.

Introduction

Within the last decade, the number of short-term assays for screening mutagenic and carcinogenic chemicals has proliferated. Representative assays include *Salmonella* mutagenesis (Ames et al. 1975), sister chromatid exchange (Stetka & Wolff 1976a, b; Kligerman 1979), micronucleus formation (Schmid 1976), DNA repair (Swenberg and Petzold, 1877) and mutagenesis (Mankovitz et al. 1974; Cole and Arlett, 1984). These test systems often measure one specific end-point in a spectrum of cellular responses. To properly integrate and interpret the results from these assays, the relationships among the responses must be determined. For example, one assay may be predictive of another, as has been suggested for the induction of sister chromatid exchanges (SCE) and mutagenicity (Wolff et al. 1977; Wolff 1978; Carrano et al. 1978). Conversely, several investigators have suggested that the induction of cytotoxicity, chromosomal aberrations, and inhibition of DNA synthesis are distinct from the induction of mutations (Peterson et al. 1979; Peterson 1980; Natarajan et al. 1984). There is, however, a strong mechanistic correlation between mutagenicity and carcinogenicity (Brusick, 1980; Newbold et al. 1980; Bartsch et al. 1983; Kaina et al. 1983; Natarajan et al. 1984). None of these studies has addressed the relationship between specific genotoxic end-points and classic toxic end-points. Consequently, correlations can not be made until studies are carried out which establish mechanistic relationships.

To monitor the aquatic environment, several genotoxicity assays which utilize aquatic animals and cell cultures, have been adapted from mammalian tests (Barker and Rackham 1979; Kligerman 1979; Walton et al. 1984; Kocan et al. 1985; Liguori and Landolt 1985). This report describes the results of an in vitro study designed to determine the relationships that exist among 1) a classical end-point, (cell death), 2) a macrolesion (anaphase aberrations) and (3) a microlesion (point mutation to ouabain resistance). The three responses studied represent a range of cellular organization: (1) cell death is indicative of nonspecific cytotoxicity, (2) anaphase aberrations detect gross damage to entire chromosomes and/or the spindle microtubules, and (3) mutations to ouabain resistance result from a heritable change in specific individual base pairs of DNA.

Materials and Methods

Cell culture conditions

BF-2 cells derived from the bluegill sunfish (*Lepomis macrochirus*), were cultured at 25°C in Liebovitz's L-15 medium supplemented with 10% heat inactivated fetal bovine serum and antibiotics. The cells were demonstrated to be mycoplasma-free by the method of Russell et al. (1975). Cells were subcultured as needed (typically 3-4 days) with .025% trypsin-0.5 mM EDTA.

Chemicals

Stock solutions of 10 mg/ml MNNG (Sigma Inc., CAS Registry #70-25-7) were made in spectrophotometric grade dimethyl sulfoxide (DMSO, Schwarz/Mann) and stored at

-30°C. Working solutions were prepared immediately prior to use by dilution in complete medium. Stock solutions more than 2 weeks old were not used. Ouabain (Sigma) was dissolved in serum-free medium to yield a 10 mM working stock, and diluted as needed.

Cytotoxicity assay

Actively dividing cells were cultured in 24-well multiple exposure trays to give an approximate density of 1 x 10^4 viable cells/well (5 x 10^3 cells/cm^2). Viability was determined by trypan blue stain exclusion (0.4%). After 24 hours, the cells in four wells were counted and averaged to provide the initial cell number (ie. t_o). The remaining wells received fresh medium containing either MNNG or 0.1% (v/v) DMSO, and exposed for an additional 96 hours. The number of surviving cells were counted using an electronic particle counter (Model ZB1, Coulter Electronics). Wells were rinsed with 2 ml of phosphate buffered saline (PBS) to remove dead, unattached cells, then the live cells were suspended with 1 ml of EDTA-trypsin solution and counted.

Anaphase aberration assay

Time course analysis: To determine the frequency of anaphase aberrations in a population of actively dividing cells, a time course of anaphase aberration induction was determined. Actively dividing cells were pipetted into Leighton tubes containing an 11 x 55 mm glass coverslip to give a density of 1 x 10^5 cells/coverslip. The cells were allowed to attach and grow for 24 hours, after which the medium was replaced with fresh medium containing 1 µg/ml MNNG, 0.1% DMSO, or complete medium. Coverslips were removed every 12 hours from the tubes exposed to MNNG, and every 24 hours from the DMSO and untreated tubes. The coverslips were placed in 3:1 methanol-acetic acid (Carnoy's fix) for 20-30 minutes, then stained for 20 minutes in 3% Giemsa in phosphate buffer (pH 6.8). Slides were coded, randomized, and the anaphase figures classified as normal or abnormal (Nichols et al. 1977) by visual examination at 250-1,000 X magnification with bright field illumination. For each dose, a minimum of 100 anaphase figures were examined on duplicate coverslips.

Dose response: The procedures for culturing, fixing, staining, and examining the cells are described above. Cells were exposed to concentrations of MNNG ranging from 0-5ug/ml of medium, 0.1% DMSO, or complete medium for 48 hours, then examined for anaphase aberrations.

Mutagenicity assay

Ouabain concentration: Three clones were isolated from both wild-type and ouabain-resistant (Oua^r) BF-2 cultures using dilute plating and cloning collars. Triplicate 100-mm petri plates were used to grow 200 cells/plate in medium containing 10^{-9} to 10^{-3} M ouabain, as well as a control containing no ouabain. After 9 days of growth, the cells were fixed, stained, and the number of colonies consisting of 50 or more cells counted. The ouabain concentration that resulted in minimal cloning efficiency for the wild-type cells, and maximal cloning efficiency for the mutant cells was selected as optimum for mutant selection experiments.

Expression time: Approximately 5 x 10^5 cells were grown in 25-cm^2 flasks for 24 hours, exposed to 1 µg/ml MNNG for 5 hours, and allowed to grow for 9 days (expression period). On day 9, 10^7 cells were removed and plated in 100-mm petri plates (2 x 10^5 cells/plate) containing 1 µM ouabain. On days 10-19 the number of mutant colonies in 5 plates (10^6 original cells) were counted.

Mutant confirmation: Mutant colonies were isolated using dilute plating and cloning collars, and allowed to grow for a minimum of 1 week (about 8 cell cycles). The cells were then tested for ouabain resistance by plating 200 cells in each of three 60-mm plates containing 1 µM (10^{-3} M) ouabain. After 9 days incubation, the cells were fixed, stained, and the number of mutant colonies counted.

Dose response: Exponentially growing cells were plated at a density of 5 x 10^5 cells/25-cm^2 flask and incubated until they reached about 10^6 cells/flask. The cells were then exposed to MNNG or 0.1% DMSO in complete medium for 5 hours. After exposure, the cells were rinsed with PBS and incubated in complete medium for an expression time of 9 days. The cells were subcultured 1:2 on days 3, 5 and 7. On day 9 the cells were detached with EDTA-trypsin and plated in medium containing 1µM ouabain for a selection time of 9 days. For each dose a total of 10^6 cells were screened for ouabain resistance (2 x 10^5 cells/100-mm petri plate). On day 13, the medium was replaced with fresh ouabain-containing medium. In addition to screening for ouabain resistance, cloning efficiency was determined for each dose by plating 200 cells in each of the three 60-mm plates and incubating in complete medium. On day 19 the mutation frequency was determined by dividing the total number of mutant colonies for each dose by the number of cells selected (10^6), corrected for cloning efficiency (usually 45-55%), and expressed as mutants per 10^6 clonable cells.

Data analysis

Data were analyzed using a one-way analysis of variance (ANOVA, two-tailed) to determine whether treatment groups were significantly different. If a significant difference was demonstrated ($P \leq 0.05$), Dunnett's multiple range test was used to identify which groups were different.

Results

Cytotoxicity assay

MNNG produced a significant decrease in cell survival at concentrations greater than 0.1 µg/ml (Table 1). Cytotoxicity remained relatively constant (25-33% of control) at low concentrations of MNNG (0.1-1.0 µg/ml), then increased dramatically above 1 µg/ml. All concentrations of MNNG produced some reduction in cell number relative to the DMSO control. The DMSO and untreated controls were not significantly different from each other.

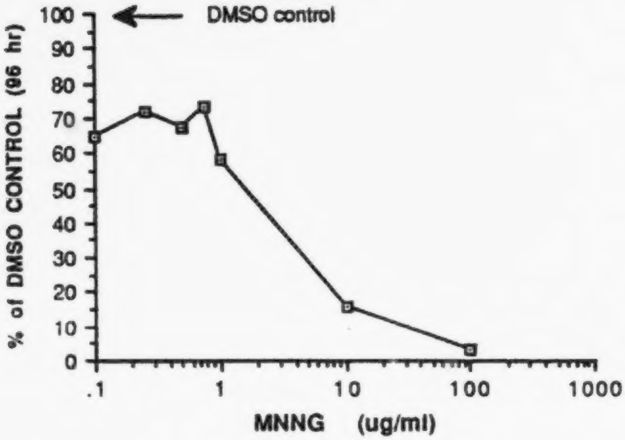

Figure 1. Survival of cells in mass culture 96 hours after exposure to MNNG. $1x10^4$ cells were initially exposed, and these increased to $1.3x10^4$ in the DMSO controls. Cell replication was inhibited below 1ug/ml and cell death occurred at >1ug/ml MNNG.

Anaphase aberration assay

Time course: After exposure to 1 µg/ml MNNG, an actively dividing population of cells displayed the maximum frequency of anaphase aberrations within 40-45 hours of exposure. Significant increases ($P \leq 0.05$) in anaphase aberrations were observed after 19 hours. The frequency of abnormal anaphases returned to control levels after 115 hours. No significant differences were observed between the DMSO and untreated cells; the frequency in both groups remained at 4-7% throughout the experiment.

Dose response: MNNG significantly ($P \leq 0.05$) increased the percentage of abnormal anaphases at all concentrations except 0.25 µg/ml. No significant difference was observed between the DMSO control and untreated cells; the frequency in both was 4-6%. Concentrations greater than 2-3 µg/ml resulted in mitotic inhibition, evidenced by a low number of mitotic cells, and resulted in increased variability.

Mutagenesis assay

Ouabain concentration: A 10^{-6}M (1µg/ml) ouabain concentration was determined to be optimum for mutant selection (Table I). This value is consistent with previous results of Kocan et al. (1981) and Mitani (1983). The mutant colonies exhibited a 10-fold increase in ouabain resistance over the controls, suggesting that the cells express an Na^+/K^+ ATPase with a decreased affinity for ouabain (Baker 1979).

Expression and selection time: The recovery of mutant cells was found to approach a maximum after five days of expression, and after eight days of growth in selective medium. However, high concentrations of MNNG (5-10µg/ml) produced enough cell mortality to require a longer expression time in order to allow the cells to recover; therefore, nine days was chosen as optimum. Selection times longer than 10 days resulted in adjacent colonies growing convergent in the control cultures, while incubation for less than 7 days resulted in colonies with fewer than the required 50 cells minimum.

Table I. **Percent cloning efficiency as a function of ouabain concentration for wild-type and ouabain-resistant clones.**

	% cloning efficiency	
Ouabin (M)	Wild-type	Oua^r
0	60	63
10^{-9}	57	72
10^{-8}	4.8	66
10^{-7}	0	55
10^{-6}	0	30
10^{-5}	0	0

Values expressed are the mean cloning efficiency of three separate clones for both wild-type and ouabain-resistant cells.

Mutant confirmation: Isolation and growth of three oua^r clones in the absence of selective pressure for at least five cell generations without loss of ouabain resistance confirmed that the cells had not been induced, but had actually undergone a heritable change in phenotype.

Dose response determination: Ouabain resistance proved to be a reliable, precise and sensitive mutagenesis marker, showing significant increases in the mutation rate at all concentrations of MNNG tested. The spontaneous mutation frequency, determined from selecting nearly 10^7 cells from DMSO control cultures, was between 0-1 mutant/10^6 clonable cells.

Relationships between assays: To examine the relationships between the assays, the responses of the assays were plotted against one another. If two genotoxic endpoints share a common mechanism their relationship would most likely be linear. Conversely, a non-linear relationship suggests a high probability that different mechanisms are responsible for the two responses. These relationships are presented in Figures 1-3.

Discussion

While there are several mechanisms that produce anaphase aberrations (Nichols et. al., 1977), the major lesion which one observers is missegregation of whole or fragment chromosomes. These lesions have been associated with developmental abnormalities in rainbow trout embryos (Liguori and Landolt 1985) and a strong correlation exists between the structural rearrangement of chromosomes, aneuploidy, and developmental abnormalities in humans (Evans 1983). Mutations to ouabain resistance, in contrast to anaphase aberrations, proceed by generally well-described mechanisms, and result in heritable, stable changes to the genotype of the organisms.

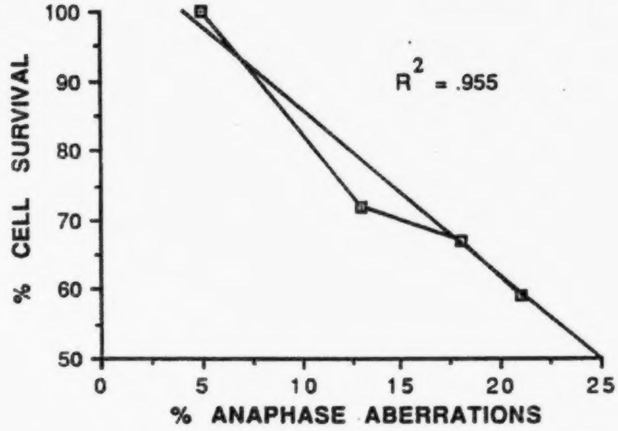

Figure 1. The linear relationship between anaphase aberrations and cell survival suggests a probable mechanistic relationship between the two events. Cells were exposed to 1ug/ml of MNNG for both end-point determinations.

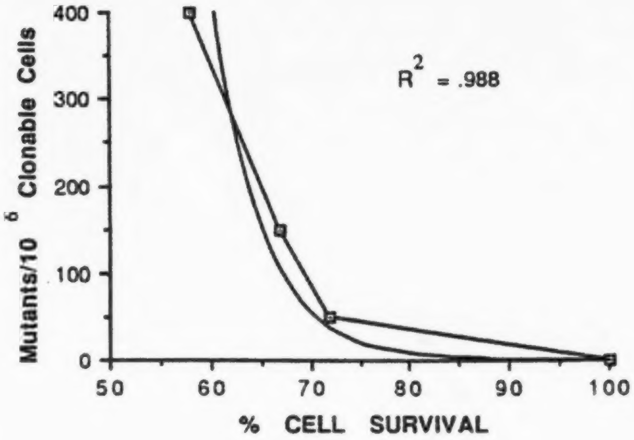

Figure 2. The relationship between cell survival and mutagenesis is described by second degree polynomial, consistent with independent mechanistic causes.

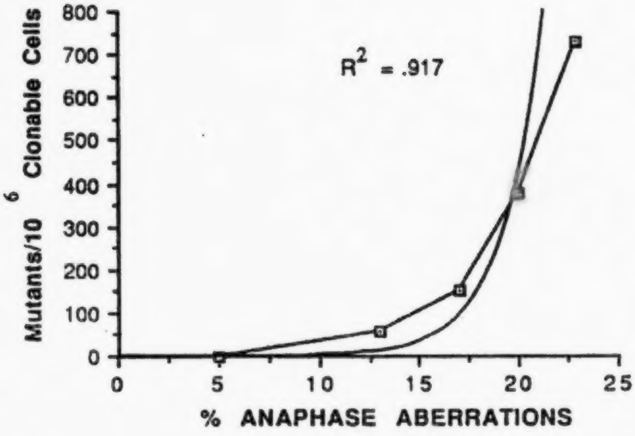

Figure 3. The relationship between anaphase aberrations and mutagenesis is exponential, indicating unrelated underlying mechanisms.

In these studies, the response observed in the mutagenesis assay remained linear over a greater concentration range than did the anaphase aberration assay. While the response for the anaphase aberration assay reached a maximum at concentrations greater than 2µg/ml, the mutagenesis assay was responsive up to 5 µg/ml. In addition, the variability of the mutagenesis assay was less than that for anaphase aberrations. The low spontaneous mutation frequency is indicative of the selective mutations that confer ouabain resistance (Baker 1979) and allows the detection of very small increases in mutation frequency. The high number of oua^r mutants produced in BF-2 cells relative to mammalian cells (Amacher and Dunn 1985) may be due to the low level of DNA repair generally found in fish cells (Walton et al. 1984).

Relationships between assays: Cytotoxicity and anaphase aberrations appear to be linearly related; as the incidence of anaphase aberrations increases, the percent cell survival decreases. This linear relationship suggests that these end-points may be mechanistically similar. In contrast, the relationships between mutagenesis and both cytotoxicity and anaphase aberrations are best represented by polynomial and exponential equations, respectively. Therefore, as anaphase aberrations and cytotoxicity increase, the incidence of mutations increases in a nonlinear fashion. These relationships are consistent with the assumption that mutagenesis is mechanistically independent of the other endpoints.

A nonlinear (exponential) relationship between cytotoxicity and mutagenesis was also observed by Mitani (1983) using goldfish-derived cells exposed to MNNG. These conclusions also agree with previous work conducted with mammalian cells, indicating the lesions responsible for cytotoxicity, inhibition of DNA synthesis, SCEs, and chromosomal aberrations are distinct from those responsible for mutations (Peterson et al. 1979; Peterson 1980; Natarajan et al. 1984). Therefore, cytogenetic assays using fish cells in culture respond to alkylating agents in a similar manner as mammalian cells, and appear to display the same types of relationships between cytotoxicity, chromosomal macrolesions, and mutagenesis.

Many more types of chemicals must be evaluated before the relationships between the assays can be firmly established, including those chemicals that require biotransformation and those that act via different mechanisms than MNNG. A more difficult task will be to show cause and effect relationships in lower vertebrates or invertebrates between genotoxic lesions and physical defects, such as those recognized in humans (eg. Down's or Kleinfelters syndrome). Because genotoxic agents act at concentrations below those required to produce classic toxic responses, such relationships may never be made. Consequently, each genotoxic response should be considered a valid toxic end-point, regardless of whether or not it has been correlated with other toxic end-points.

Acknowledgements

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DNA-CARCINOGEN ADDUCTS IN AQUATIC ORGANISMS. B.P. Dunn, B.C. Cancer Research Center, 601 W. 10th Ave. Vancouver, B.C., Canada (604-877-6010)

Extended Summary

The occurrence of cancer in wild fish populations in many cases has been linked the occurrence of carcinogens in the aquatic environment. In the best studied examples, epidemiological data on liver tumors in brown bullheads and in certain marine flatfish species have suggested a link to carcinogens of the polycyclic aromatic hydrocarbon (PAH) class. PAH have also been shown to be capable of inducing tumors in experiments using captive fish. This class of carcinogens is thought to act by being metabolized to reactive electrophilic species which covalently bind to DNA and induce DNA mutations. In laboratory experiments, such binding is normally measured by using radioactively labelled carcinogens.

Recent developments in methodology have allowed the direct measurement of the covalent binding of non-radioactive carcinogens from environmental exposures to DNA. Useful techniques include fluorimetric, immunoassay, and radioactive-postlabeling methods. We have used 32P-postlabeling methodology for measuring DNA-carcinogen adducts in aquatic organisms exposed to PAH.

Elevated levels of aromatic DNA-carcinogen adducts were found in the livers of brown bullheads from areas known to be contaminated with PAH, and in which the wild bullhead populations suffer from an elevated level of liver cancer. Elevated levels were also found in starry flounders from a PAH contaminated inlet. In contrast, in a limited survey there was no difference in the levels of adducts in mussels from clean and carcinogen contaminated areas. The failure to detect pollution related adducts in mussels most probably reflect the general inability of shellfish to metabolize PAH to DNA-reactive materials.

For studies of aquatic carcinogen contamination and of the etiology of cancer in wild fish populations, the measurement of DNA-carcinogen adducts may prove highly useful. The methodology has the advantage that it measures the biologically effective dose of carcinogens to a given species and tissue, rather than just the environmental concentration. The methodology needs relatively few fish samples, compared to surveys of tumor levels in wild fish populations. The major complication of the methodology is that the "baseline" levels of DNA-carcinogen adducts as measured by 32P-postlabeling in organisms from clean areas do not appear to be zero. The origin of these "background" adducts is currently unknown, but they appear to be species and tissue specific, and may be related to spontaneous DNA damage caused by reactive species produced during normal cellular metabolism. The occurrence of "background" adducts may limit the ability of 32P-postlabeling to detect adducts induced by relatively low levels of environmental pollution.

SUBLETHAL TOXICITY OF PULP MILL EFFLUENTS EVALUATED BY THE MEASUREMENT OF METALLOTHIONEIN AND MIXED FUNCTION OXIDASE INDUCTION IN THE LIVER OF RAINBOW TROUT.

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INTRODUCTION

In the Saint-Lawrence river, they are several pulp mill industries who reject their effluents. These effluents are complex mixtures which are organic (aromatic hydrocarbons) and inorganic (heavy metals) in nature. It is relevant for the Saint-Lawrence River Action Plan to evaluate their subletal effects in order to provide a better understanding of the ecotoxicological impact of pulp mill effluents on aquatic organism. Heavy metals like cadmium (Cd), copper (Cu), mercury and zinc (Zn) are known to induce the synthesis of a metal binding protein in the liver of fish, rich in cysteine, nammed metallothionein (MT). Their function is supposed to be homeostatic regulation and detoxification of essential and non-essential metals. The sequestration of metals by MT depends on the respectives affinities of the metals for the protein and their abondance. This sequestration of metals is implicated in the protection mecanism by decreasing the free form of the toxic metals, thus diminishing their toxic effects (Hamilton and Mehrle, 1987; Roch and Mc Carter, 1984). Organic compounds are known to induce a hemoprotein called cytochrome P-450. This protein has a mono-oxygenase acitivity, which can oxidize foreign and endogenous compounds into a more hydrophilic metabolite (phase 1 reactions). This metabolite can be directly or indirectly, i.e. be conjugated by sugars or peptides (phase 2 reactions), eliminated (Donald deBethizy and Hayes, 1989). The metabolites are often more electrophilic and their toxicities could be elevated or diminished depending upon the reactivity of the metabolites toward sensible sites (i.e. proteins and nucleic acids) in the tissu. The aim of this study is to validate the use of these 2 biochemical parameters in order to evaluate the organic and inorganic stress of the effluent to the fish, and to better understand their subletal effects. These biochemical parameters would permit a better diagnostic of the toxicological impact of the effluents on the fish which are released in our environment.

METHODS

A static 96 h - bioassay was used in this study. A preliminary bioassay using 4 fishes per dilution of the effluent was done in order to estimate the lethality of the effluent, the dilution were made in the range where no mortality occurred. Rainbow trouts (Onchoryncus mykiss) were exposed for a 96 h period at 15°C with several dilutions of the pulp mill effluent (5.6, 10, 18 %). After this incubation period, the trouts were removed and dissected to obtain the livers. These were rinced in iced-cold tris-acetate buffer, 25 mM pH 7.5, containing 250 mM sucrose and 1 mM dithiothreitol. The livers were homogeneized with a potter elvehjeim tissu grinder and centrifuged at 10 000 x g for 20 min at 4°C. The supernatant (S_{10} fraction) was kept on ice until analysis. The S_{10} fractions were analyzed for MT content according to the silver saturation assay (Sheuhammer and Cherian, 1986) with the modification of Gagné et al. (1990). The cytochrome P-450 content were analyzed by the CO-oxidize versus CO-reduce spectrum method in the presence of phenazine ethosulfate and ascorbic acid which corrects against spectral interference of hemoglobin (Johannesen and De Pierre, 1978). The activity of 7-ethoxyresorufin deethylase activity was determined in the S_{10} fraction according to the method of Prough et al. (1977). In the assay buffer, 10 uM of dicoumarol was added to inhibit the cytosolic quinone oxydoreductase which can transforme the product (i.e. 7-hydroxyresorufin) to a non-fluorescent product (Nims et al., 1984). Protein content was determined by the method of Bradford (1976). For each conditions, 10 fishes were used and the experiments were repeated 4 times (n=4). The data were subjected to an analysis of variance and critical difference between controls and treatments was evaluated by the Dunnett t test. Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The estimated LC_{50} of the effluent was in the range of 70 - 100 % after an incubation period of 96 h. Nevertheless, the levels of MT in the liver of the fishes were significantly increased at 18 % dilution of the effluent (Fig. 1). This induction indicates that metals are present in the effluent and are bioavailable to the fishes. The induction of MT indicates that the fishes are stressed by the presence of metals.Indeed, the effluent contained substancial amounts of metals: 728 ng\mL of Zn, 14 ng\mL of Cu, 1000 ng\mL of aluminium and 6 ng\mL of lead. These metals are known to be good inducers of MT exept for aluminium (Cherian and Nordberg, 1983). The levels of cytochrome P-450 in the liver of fish exposed to the effluent decreased significantly at 10 and 18 % dilution of the effluent (Fig. 2). Although the total organic carbon content in the effluent was relatively high (350 mg\L), no induction of the protein was detected. However when the specific activity of EROD was measured, we detected no significant increase in the enzyme activity with the concentration of the effluent when the specific activity was obtained by normalisation the enzyme activity with the protein content in the S_{10} fraction (Fig. 3). When the specific activity of EROD was obtain by normalization by cytochrome P-450 levels (i.e. pmole of product formed\min\pmole of cytochrome P-450) significant increase in the activity was detected (Fig. 4). The reason for the decrease in the levels of hepatic cytochrome P-450 is unknown but it is known that metals like Cu, Cd and Zn could cause a degradation of cytochrome P-450 (Eaton et al.,1980). This degradation is associated with the induction of an enzyme named heme oxygenase wich is associated with the catabolism of hemoproteins and is inducible by certain heavy metals. When we evaluated the activity of heme oxygenase (result not shown), we detected significant induction at 10 and 18 %, dilutions where a decrease of cytochrome P-450 is detected. In conclusion, the utilisation of MT and MFO induction are usefull end points in assessing the subletal toxicity of pulp mill effluents. However, the determination of total cytochrome P-450 alone as a parameter of organic stress must be interpretated with caution when assessing the toxicity of effluents.

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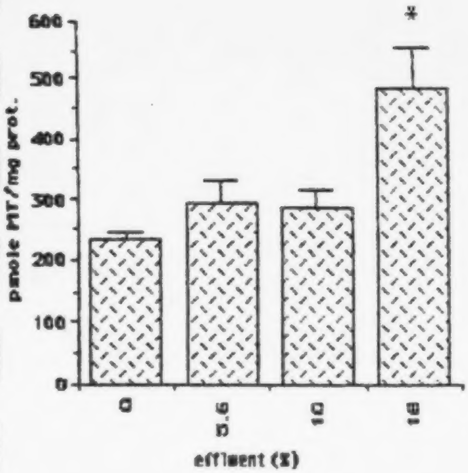

Figure 1 : Hepatic levels of MT in trouts exposed to in the effluent.
The levels of MT were determined with the silver satu-assay. The experiments were repeated 4 times.
The data is expressed as the mean with the standard the error. * significant at $p<0.05$.

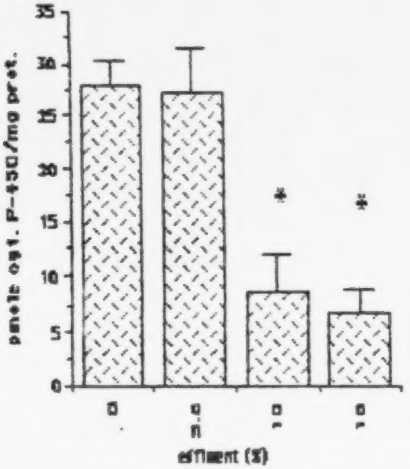

Figure 2: Hepatic levels of cytochrome P-450 in trouts exposed to the effluent.
The levels of total cytochrome P-450 were evaluated as described in Methods. The experiments were repeated 4 times. The data is expressed as the mean with the standard error. * significant at $p<0.05$.

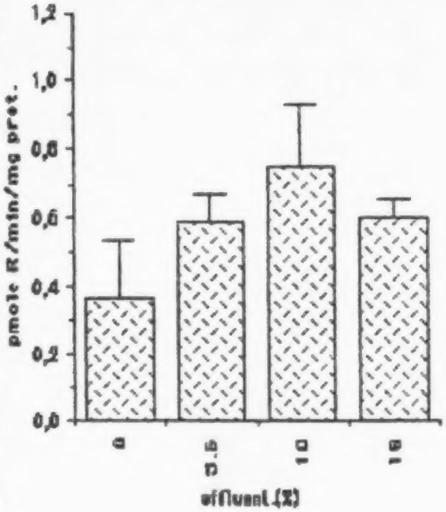

Figure 3: EROD activity in the liver of trouts exposed to the efluent.
The specific activty is expressed as pmole of resorufin formed per min per mg protein in the S_{10} fraction. The experiments were repeated 4 times and the data is expressed as the mean with the standard error.

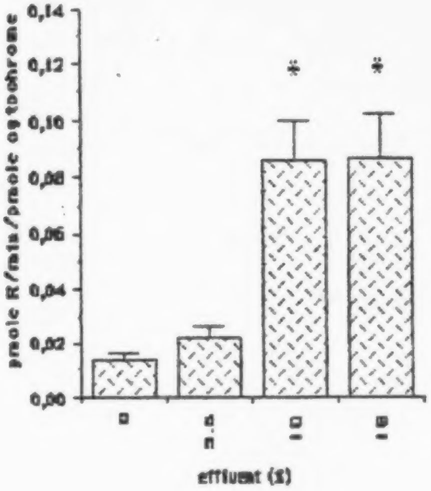

Figure 4: EROD acitvity in the liver of trouts exposed to the effluent.
The specific activity is expressed as pmole of resorufin formed per min per pmole of cytochrome P-450. The experiments were repeated 4 times and the data is expressed as the mean with standard error. * significant at $p<0.05$.

A New Microbial Bioassay for Screening of Sediment Mutagenicity

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A new methodology for a standardized, microbial bioassay has been developed and adapted to determine mutagenicity in organic extracts from marine sediment samples. The test is based on *de novo* enzyme synthesis of β-galactosidase in a rough mutant strain of *E. coli* (PQ37). Linkage of the β-galactosidase gene to the SOS operator gene permits production of the enzyme whenever the native SOS repair system is activated as a result of genotoxic agents causing lesions in DNA. Even cells which are not able to divide and produce colonies are capable of indicating positive results *via* SOS induction. The enzyme is readily detected by chromogen reaction and results are read either visually or by microtitration plate photometer. Promutagenicity of sediments is indicated by results from splits of samples, run with an S-9 liver extract. This bioassay has been shown to have 90 percent concordance with Ames test results for over 250 chemicals.

Because the bioassay is conducted in a matter of hours in 96-well microtitration plates, this new tool offers a high-volume, low-cost alternative for the rapid screening of sediment mutagenicity and promutagenicity.

Marine pollution assessment work is limited by the availability of rapid, inexpensive tests of biological injury, in particular, mutagenic injury. Methods are needed so that the potential biological threat of contaminated sediments can be quickly and accurately surveyed to estimate the possible scale and extent of damage, and the location of sites which should be more intensively examined. Methods for identification of potential mutagenic impacts are particularly in need of further development.

Numerous chemicals known to be mutagenic have also been shown to be carcinogenic (Ames *et al.* 1973). These compounds may be either reactive electrophiles, or can be metabolized to reactive species, which interact with nucleophilic centers of DNA (Miller and Miller 1981). The widespread chemical detection of known carcinogens in sediments, plus their metabolic by-products in fish, clearly indicates their presence and bioavailability in the marine environment (Krahn *et al.* 1984; Malins *et al.* 1982). And, identification of DNA adducts in these fish confirms the genotoxic nature of these compounds (Varanasi *et al.* 1989). However, complete chemical analysis of environmental samples to determine the presence of hundreds of potentially carcinogenic and mutagenic compounds is very rarely done. More often, bioassays are used to assess the potential for mutagenic impacts, but rarely in a field survey mode. Microbial assays of sediments offer the opportunity to conduct relatively inexpensive and rapid surveys of potentially genotoxic sites.

Microbial bioassays have been widely employed to identify mutagenic agents. The Ames test has been pivotal in the increased use of bacterial assays, and is now the most extensively used and validated bacterial short-term test (Ames *et al.* 1975; Bitton and Dutka 1989). Application of the Ames test with marine sediment samples has its limitations. For instance, its 2-day incubation period before counting mutant colonies mandates aseptic samples which are sufficiently nontoxic to allow development of colonies.

This paper presents a methodology for a new application of a short-term microbial assay, the SOS Chromotest™. This bioassay determines mutagenicity and promutagenicity of organic extracts of marine sediment. The SOS Chromotest™ has been used to assess the genotoxicity of hundreds of drugs and foodstuffs, and recently has been utilized in environmental studies (Dutka *et al.* 1987; Dutka *et al.* 1986; Smith 1988; Xu *et al.* 1987). This bioassay offers numerous practical advantages for large-scale, rapid screening of mutagenicity of marine sediments.

The SOS Chromotest™ is based on the ability of toxicants to penetrate the cell wall of a highly permeable, mutant strain of *Escherichia coli* (PQ37) and interact with general material. An operon fusion in this strain of *E. coli* has linked *lacZ*, the structural gene for the enzyme β-galactosidase, under control of the *sfiA* gene. The *sfiA* gene is one of many unlinked genes in an inducible network, referred to as the SOS system (originally named after the international distress signal), which repairs DNA damage. This was the first regulatory network induced by DNA damage to be recognized and described in detail at the molecular level (Walker 1984). The SOS system is held in a repressed state in normal, uninduced cells. When a cell's DNA is damaged or its DNA replication is hampered, an induction signal is generated. This signal activates the SOS DNA repair system. In the PQ37 strain, *via* the *lacZ-sfiA* fusion, this induction signal triggers production of β-galactosidase as well. Activity of the induced β-galactosidase is detected by its reaction with a chromogenic substrate. Results can be read qualitatively with the naked eye or quantitated using microtitration plate spectrophotometers (ELISA readers). Other genetic alterations of this *E. coli* strain enhance sensitivity of the assay. A *uvrA* mutation creates a deficiency in excision repair thus increasing response to certain agents. An *rfa* mutation results in a lipopolysaccharide deficiency which allows greater diffusion into the cell.

To evaluate the performance of the SOS Chromotest™ with marine environmental samples, bioassays of organic extracts of sediment contaminated from an oil spill were conducted. To evaluate the feasibility of the SOS Chromotest™ as a real-time screening tool, tests were conducted onboard ship, with final results reported within 24 hours.

Materials and Methods

Surficial sediment samples used for this field evaluation had been contaminated to varying degrees with crude oil following an oil spill. Samples tested were collected in the intertidal zone, 3-m, and 6-m depths from 16 sites tested and extracted as described by Buchman (this volume). The extraction process produced a DMSO extract which was used in the bioassays. Samples were collected at sites where the likelihood of any other form of anthropogenic contamination was negligible.

SOS Chromotest™ bioassays were conducted in sterile, disposable, 96-well ELISA microtitration plates (8 rows by 12 columns). Three plates of samples (12 samples) were run during each batch. Each sample was run in duplicate dilution series with and without S-9 activation. Inclusion of S-9, a crude liver extract from PCB-induced male rats, is intended to mimic the *in vivo* bioactivation of promutagens. Each batch included assays of two carcinogens as positive controls - 1 μg/mL 2-amino-anthracene (2AA) in 10% DMSO in saline served as the positive control with S-9 activation, and 10 μg/mL 4-nitroquinoline 1-oxide (4NQO) in 10% DMSO in saline served as the control without S-9 activation.

A rough mutant of E. coli, PQ37, was supplied by Orgenics in lyophilized form. Under aseptic conditions, the bacteria were rehydrated in 10 mL of growth medium (1% Bacto Tryptone, 0.5% Bacto yeast extract, 1% sodium chloride), supplemented with 20 μg/mL ampicillin, and incubated for 16 hours at 37°C. Optical density (OD) of the suspension at 600 nanometers (nm) was read. This OD value was used to prepare two 20 mL solutions of equal cell density using fresh growth medium, one without and one with S-9. The S-9 used was a lyophilized mixture containing approximately 8 mg protein from male Sprague Dawley rat liver, NADP (15.3 mg), G-6-P (7.05 mg) in a 0.2 M TRIS buffer (pH 7.4), with $MgCl_2$ and KCl as stabilizing agents. It was reconstituted in 5 mL iced, double-distilled water and held in an ice bath for 10 minutes before use.

ELISA plates were prepared to accommodate 12 samples and 2 positive controls among three plates. One well, which served as a machine blank, received 10 µL of standard diluent (10% DMSO in saline) and 100 µL of the growth medium without bacteria. Two sets of wells contained serial dilutions of the positive controls. The 4NQO control dilution series started at 10 µg/mL and the 2AA control started at 100 µg/mL. Remaining wells were divided for dilution series of test samples. The last row of wells in every test set received only standard diluent and served as negative control. Each test well received 10 µL of diluted sample extract. Serial dilutions for each sample were prepared in microfuge tubes using GC syringes. After samples were dispensed, 100 µL bacterial mixture were added by multichannel digital pipette and the plates were incubated for 2 hours at 37°C.

Following incubation, 100 µL of chromogenic mixture were added to every well. The chromogenic mixture contains lysing agents (detergents and solvents) in a pH 9.0 Tris buffer system and bromo chloro indoxyl β-D-galactoside (BCIG). BCIG, the substrate for β-galactosidase, will develop into a deep blue color in bacterial suspensions whose SOS systems have been induced. The plates were then returned to the incubator for an additional 2 hours after which time the OD at 615 nm was read in an ELISA spectrophotometer.

Because sediment extracts could impart a toxic action, in addition to mutagenesis, the viability of each test bacterial suspension was checked *via* an alkaline phosphatase reaction. Test solutions of high general toxicity could inhibit protein synthesis and lead to false negatives. Following BCIG color development, 50 µL of *p*-nitrophenyl phosphate disodium (PNPP) solution was added. In healthy cells, PNPP, an alkaline phosphatase substrate, will develop a deep yellow color (read at 415 nm).

The presence of oil in sediment extracts was analyzed by fluorescence at 310 nm EX and 370 nm EM. The instrument was blanked with methylene chloride and calibrated against standard dilutions of crude oil extracts for each sample run.

All solvents were HPLC grade. The lyophilized S-9 mixture was from MolTox™. All other reagents were supplied in kit form from Orgenics. All reagents were stored at 4°C.

Results

In the SOS Chromotest™ bioassay, the operon for β-galactosidase has been linked to an SOS operator gene in the PQ37 strain of *E. coli.* Objective determination of color development in test solutions is then a result of the activity of β-galactosidase and indicates whether the SOS system has been induced in direct response to DNA damage or inhibition of DNA replication.

In this field evaluation of the SOS Chromotest™, 44 extracts of marine sediment samples were assayed for mutagenicity and promutagenicity.

None of 44 samples tested displayed direct mutagenicity. Proper performance of the bioassay to detect direct mutagens was verified in every batch of samples by development of color in the positive control, 4NQO. Assays of promutagenicity were hampered by poor performance of the S-9 activation mixture, as indicated by lack of reaction in the positive control, 2AA. Only one batch of five samples provided acceptable results, relative to the control. All five samples in this batch indicated significant mutagenic activity following activation of promutagenic compounds present in the sample extracts. These samples were among the most heavily oiled, as indicated by fluorescence. Slight activation was displayed in the positive control with one other batch of S-9; but, it was not great enough to be considered substantially different from the background level of activity observed in the negative controls. Results of test samples in this batch were suggestive of promutagenicity, but the level of activation observed in the positive control was too weak to adequately verify performance of the bioassay.

Assays of alkaline phosphatase activity were used as indicators of the general biosynthetic health of the bacterial suspensions. Samples that failed to indicate significant β-galactosidase activity were subsequently checked for viability by addition of PNPP, an alkaline phosphatase substrate, and scanned 30 minutes later for yellow color development. These analyses verified that test solutions were not significantly toxic as tested in all 44 samples.

Discussion

The SOS Chromotest™ is designed to measure a primary and early response of *E. coli* to genotoxic damage. Even cells that are incapable of dividing respond enzymatically and thus are capable of generating positive results. Sensitivity of the bioassay is enhanced by engineering a strain of *E. coli* that is lipopolysaccharide deficient for greater diffusion into the cell and cannot accomplish excision repair and hence mask DNA damage.

Though a battery of genotoxicity tests are recommended by regulatory agencies worldwide (Brunsick 1982), limited time and rising costs of environmental assessments have rendered extensive genetic toxicity and animal testing of environmental samples prohibitive. However, rapid and inexpensive bacterial tests offer an option for increasing the ability to identify potential problem areas deserving further attention. SOS Chromotest™, offers many practical benefits as a rapid screening tool. This bioassay was successfully used by Xu, along with Microtox™, to evaluate sediment samples from two freshwater ponds (Xu *et al.* 1987). Dutka et al. used SOS Chromotest™ on water extracts of sediment samples in a battery of tests to prioritize 51 sites in Lake Ontario for remedial action or further investigation (Dutka *et al.* 1986). This battery-test approach was later applied to samples from 40 sites in Lake Erie and the Detroit River (Dutka *et al.* 1987).

In this field trial of the SOS Chromotest™ using organic extracts of marine samples, mutagenicity of sediments contaminated by crude oil was assayed. Genotoxicity of this crude oil, either fresh or weathered, has been demonstrated elsewhere with the Ames Mutatest (Sheppard *et al.* 1983). The ability of biota to uptake and process mutagens from crude oil has also been demonstrated. Kadhim (1984) reported mutagens in mussels and limpets capable of inducing both frameshift and base-substitution mutations up to one year following an oil spill. Similar findings of uptake and bioactivation of crude oil constituents in fish have been reported (Malins *et al.* 1980; Walton *et al.* 1987).

No direct mutagenic activity was observed in this field trial. Though activation of promutagens was less than fully satisfactory, limited results did indicate the presence of promutagens in samples which were highly oiled. This field trial demonstrates the applicability of the SOS Chromotest™ to marine sediment extracts.

The SOS Chromotest™ shows promise as a rapid, inexpensive screening tool for genetic toxicity. One technician can easily test as many as two dozen samples per day for an estimated cost of $40 U.S. (excluding extraction). Further evaluation of this mutagenicity bioassay should be conducted on samples from a variety of contaminated sites.

Acknowledgments

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A MULTI-FISH SPECIES EVALUATION OF DNA ADDUCTS AS AN INDICATOR OF EXPOSURE TO GENOTOXIC CONTAMINANTS. J. E. Stein, W. L. Reichert, and U. Varanasi, E. C. Division, Northwest Fisheries Center, NOAA Fisheries, 2725 Montlake Blvd. E., Seattle, WA, USA (206-442-4638).

The binding of a chemical carcinogen to DNA is believed to be an essential early step in chemical carcinogenesis, thus DNA-xenobiotic adducts have received considerable attention as an indicator of exposure of feral fish to genotoxic compounds. In the present study a wide range of marine fish species were collected from reference and contaminated areas in coastal US waters and levels of DNA-xenobiotic adducts were measured using the ^{32}P-postlabeling assay, which is a highly sensitive method that detects a wide range of xenobiotic compounds bound to DNA. The results showed that DNA-xenobiotic adducts were detected in all fish species from clearly contaminated sites, and that adducts levels were greater in these fish than in fish from reference sites, which had levels of adducts near detection limits. These results substantiate that DNA-adducts detected using the ^{32}P-postlabeling assay are reflective of contaminant exposure, and suggest that there is negligible species-specificity in the use of this assay for assessing exposure of feral fish to genotoxic contaminants.

RELATIVE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE BY 2,3,7,8-TCDD AND TWO COPLANAR PCBs IN RAINBOW TROUT

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SUMMARY

Relative aryl hydrocarbon hydroxylase (AHH) induction potencies of 2,3,7,8-TCDD, 3,3',4,4'-tetrachlorobiphenyl (IUPAC congener 77) and 3,3',4,4',5-pentachlorobiphenyl (IUPAC congener 126) were determined in rainbow trout (*Oncorhynchus mykiss*). Trout were injected intraperitoneally with chemicals dissolved in corn oil, and livers were sampled 72 hours after injection. The ED50 for AHH induction in TCDD-injected fish was estimated as 0.005 μmol/kg (1.5 μg/kg). The ED50s for PCB congeners 126 and 77 were 1.0 μmol/kg (330 μg/kg) and 2.2 μmol/kg (665 μg/kg), respectively. 2,3,7,8-TCDD equivalents, or AHH toxic equivalent factors (AHH-TEFs), were determined as the ratio of the TCDD ED50 to the ED50 for each PCB congener. AHH-TEFs for congeners 126 and 77 were 0.005 and 0.002, respectively. Toxic equivalent quantities for AHH induction (AHH-TEQs) were calculated by multiplying the AHH-TEF for each chemical by its concentration in fish at environmental levels. AHH-TEQs, based on TCDD and coplanar PCB concentrations in Lake Ontario rainbow trout, were 2 to 3 fold greater for the coplanar PCBs than TCDD.

Baseline AHH activity in control (corn oil) treated fish was dependent on the time of year, ranging from 3.0 ± 0.5 pmol/min/mg in December 1989 to 18 ± 3.1 pmol/min/mg in May 1990. The AHH induction experiment was repeated for TCDD and congener 77 in July 1990. Lower doses of each chemical were required to induce the same AHH activity in the summer experiment when compared to the previous (winter) experiment ($p < 0.05$). "Summer" ED50s for AHH induction were estimated as 0.003 μmol/kg and 0.3 μmol/kg for TCDD and congener 77, respectively. The relative and absolute AHH induction potencies of TCDD, congener 126, and congener 77 in both experiments were similar to those reported for *in vivo* studies in rats, and indicate that rainbow trout have similar sensitivity to TCDD and coplanar PCBs as rats.

Liver samples from fish intraperitoneally injected with a range of doses of coplanar PCBs were analyzed by HRGC-ECD. There was a linear relationship between nominal i.p. dose and liver concentrations of congener 126 ($r^2 = 0.93$) and congener 77 ($r^2 = 0.84$). The results indicate that approximately 3-6 percent of the nominal i.p. dose is accumulated in liver tissue by 72 hours post-injection. When liver concentrations of PCBs are related to AHH activity, lower concentrations of congener 126 than congener 77 induce a similar AHH response in rainbow trout. Maximum AHH activity was observed at liver concentrations of approximately 200 nmol/kg (120 μg/kg) of congener 126 and 400 nmol/kg (70 μg/kg) of congener 77.

The adverse effects of TCDD and related compounds on growth, condition, and reproduction of fish is of great concern. Elevation of AHH activity has been proposed and utilized as an environmental monitoring tool for fish exposed to these contaminants. The results of this study suggest that in contaminated aquatic systems, certain PCB congeners may have greater toxicological significance to fish than TCDD.

MICRONUCLEUS ASSAYS IN PERIPHERAL BLOOD OF RAINBOW TROUT: TIMING OF RESPONSE AND CHEMICAL MUTAGEN SENSITIVITY

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ABSTRACT

The spontaneous frequency of micronuclei in fish peripheral blood is low and stable. With chronic exposure to x-radiation or aqueous trimethylphosphate, a dose-dependent elevation of micronucleus frequency is observed. The time to response is age/size-dependent, 7 to 14 days for two-inch fish, and 21 to 28 days for six-inch fish. Significant elevation of response was observed at 100 rads/day x-radiation and 2 g/L trimethylphosphate. Administration of benzo(a)pyrene in food did not produce a significant response.

RÉSUMÉ

La fréquence spontanée de micronuclei dans le sang de poisson est faible et stable. Une augmentation de la fréquence de micronuclei est observable lors d'une exposition à un niveau chronique de rayons-x ou d'une solution de triméthylphosphate. Le temps de réponse dépend de la taille (âge du poisson. Ce temps est de 7 à 14 jours pour des poissons de 2 pouces et de 21 à 28 jours pour des poissons mesurant 6 pouces. Une augmentation significative de la fréquence fut observée a 100 rads/jour de rayons-x et de même à 2 g/L de triméthylphosphate. L'addition de benzo(a)pyrène à la nourriture des poissons n'a produit aucune réponse significative.

INTRODUCTION

Interest has grown in recent years in cytogenetic studies in fish as a means of monitoring aquatic pollution and potential genetic hazards of contaminated water for man. Studies of genetic end-points in fish not only provide a valid indication of genetic damage related to aquatic contaminants, but also, because of the comparable metabolic activation systems of fish (Payne 1976), provide results which may be more pertinent to man than are the bacterial mutation assays. Cytogenetic endpoints have, in addition, the advantages of all short-term tests, those of speed and cost-effectiveness. In contrast to investigations of cancer incidence in native fish, cytogenetic studies are both rapid and cost-effective.

Cytogenetic studies have been performed in both freshwater and marine fish; most have been carried out using the mudminnow, either *Umbra limi* in North America and Japan or *U. pygmaea* in the Netherlands, and cytogenetic abnormalities have been correlated with polluted sites. The original protocol for analysis of chromosomal aberrations (Kligerman et al. 1975) has been adapted to rainbow trout (Al-Sabti 1985). The protocol was later modified to permit the analysis of sister-chromatid exchanges (SCE) (Kligerman 1979). These are exchanges of genetic material within the chromosome that are enhanced by genotoxic agents. However, the mudminnow is one of the few known species with sufficiently large chromosomes for this type of assay to be practical.

One of the most daunting problems to face investigators of chromosome breakage has been the poor quality and paucity of the metaphase figures which can be obtained from fish tissues. This hampers the analysis of chromosomal aberrations and of SCE, the former more so as aberrations occur less frequently than SCE and, therefore, more cells must be scored to detect increases which are statistically significant.

The micronucleus assay overcomes this problem entirely because chromosome breakage is analyzed in interphase cells. Micronuclei are formed when a cell containing a chromosome aberration divides. Acentric fragments not incorporated into daughter nuclei after cell division appear at the subsequent interphase as micronuclei. The newly formed (polychromatic) erythrocytes (PCEs) are distinguishable cytologically from the mature (normochromatic) erythrocytes (NCEs) in both mammals (Heddle et al. 1984) and fish (Dinnen et al. 1988). Since PCEs rapidly mature into longer-lived NCEs, the smaller PCE pool is rapidly saturated with micronucleated cells under chronic exposure to mutagenic agents, providing an index of recent mutagen exposure. The larger NCE pool is saturated more slowly, providing an index of long-term mutagen exposure. While the timing of pool saturation (peak response) is well-established for mammals, it has not been well-documented for fish.

Micronuclei have been induced in fish peripheral erythrocytes by ethyl methanesulfonate (EMS) in aqueous solution (Hooftman and deRaat 1982). Das and Nada (1986) have observed the effect in response to pulp and paper mill effluents. The effect can be measured easily in a wide variety of fish species, regardless of chromosome size, but has not yet been correlated with occurrence of fish neoplasia in the environment. Attempts to correlate neoplasia with micronucleus frequency using adult wild fish (e.g., Smith and Ferguson 1985) may be confounded by low erythropoietic activity, hence low micronucleus frequencies in older fish. Thus, our objective was to investigate fish age/size effects on timing and sensitivity of response, as well as to compare responses to a number of different mutagens.

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*) were acquired from Aquafarms Inc. in two size classes (2 inches and 6 inches) and acclimated for seven days prior to experimental use. Mutagen treatments included x-irradiation (50, 100, 400 rad/d), trimethylphosphate in water (1, 2, 4 g/L), and benzo(a)pyrene in food (10, 100, 1,000 g/kg food). Trimethylphosphate (TMP) is a weak directly acting mutagen (Connor 1979), while benzo(a)pyrene (BP) is a weak indirect mutagen requiring metabolic activation.

Irradiation methods follow Dinnen et al. (1988). The TMP solutions were changed every three days (static exposure). Trout food was treated by impregnation with BP in an acetone: corn oil carrier. Control food was treated with carrier only. Water was replaced every 12 hours, and excess food removed from the tank after feeding. Exposure and control tanks were maintained at 15±1°C. Chemical exposures (28 days) involved small fish only.

Age/size effects were investigated with radiation treatment at a single dose level (100 rad/2d), by treatment of large and small fish on alternate days. Control fish were handled, but not exposed over the 84-day experimental period. Tank temperature was maintained at 11±1°C.

Four fish from each treatment group were sacrificed at each sampling period for micronucleus scoring in peripheral blood. Sampling occurred every two to three days initially, and every seven days after the first week of treatment. Blood was obtained by severing the caudal peduncle and a smear was prepared on a slide for each fish.

The trout to be sampled were first weighed on a Mettler PC 4400 top-loading balance, then anaesthetized in a 10 g/L solution of MS-222 (3 amino-benzoic acid, 4 ethyl ester). After five to ten seconds, the tail was severed, a drop of blood placed on a slide and evenly spread. After sampling, the slides were allowed to air-dry, fixed in 100% methanol for 15 minutes, and then stained in 5% Giemsa in 1M/15 Sorensen's buffer for 15 to 20 minutes. Following staining, the slides were rinsed once with buffer and once with double distilled water, air-dried and mounted with DPX mountant.

After randomizing and coding the slides, 500 monochromatic erythrocytes (NCEs) and 500 polychromatic erythrocytes (PCEs) per slide were scored for micronuclei under oil immersion at 1,000 X magnification. The number of micronucleated cells of each type was recorded. The number of NCEs observed among the first 100 PCEs was also recorded. From these data, the NCE:PCE ratio, and the proportions of micronucleated cells among the PCEs and NCEs, were calculated at each sampling time.

RESULTS AND DISCUSSION

Micronucleus (MN) frequencies in PCEs of small fish were strongly related to radiation dose rate (Figure 1). The MN response was statistically significant at 7 days (400 rad/d) to 9 days (100 rad/d). A similar response time (7 to 14 days) was observed with a fractionated dose of 100 rad/2d (Figure 2). A plateau response (2.4 to 3.6%) was achieved after about 35 days.

Micronucleus frequencies in NCEs of small fish were only marginally elevated at 0.6 to 0.8% compared to the spontaneous frequency of 0.2%. With a marginal response, it is difficult to delineate a plateau region.

A marginal growth effect is suggested at 100 rad/2d (Figure 3). To place this dose in perspective, acute radiation LD50s for fish range as low as 1,000 rad (Auerbach 1976) and chronic growth effects have been reported at doses as low as 10 rad/d (Hershberger et al. 1978).

The time to response in large fish is greater than in small fish. The MN response in PCEs of large fish was statistically significant at 28 days, as compared to 7 days in small fish. This can be explained by a lower rate of erythropoesis, and hence MN formation, in larger/older fish. However, the plateau response level is not size/age dependent. It simply takes more time to influence and saturate the PCE pool.

The first suggestion of significant response in the NCEs of large fish occurred on day 82, at the end of the experiment (Figure 4). Thus, a plateau response level cannot be defined.

Chronic exposure to TMP produces a dose-dependent increase in MN frequency in PCEs of small fish (Figure 5). The MN response was statistically significant at 7 days (4 g/L) to 14 days (2 g/L). Thus, the timing of response is similar whether physical or chemical mutagens are used.

Figure 1
Micronuclei in PCE's of Two Inch Fish
(50-400 RAD/Day)

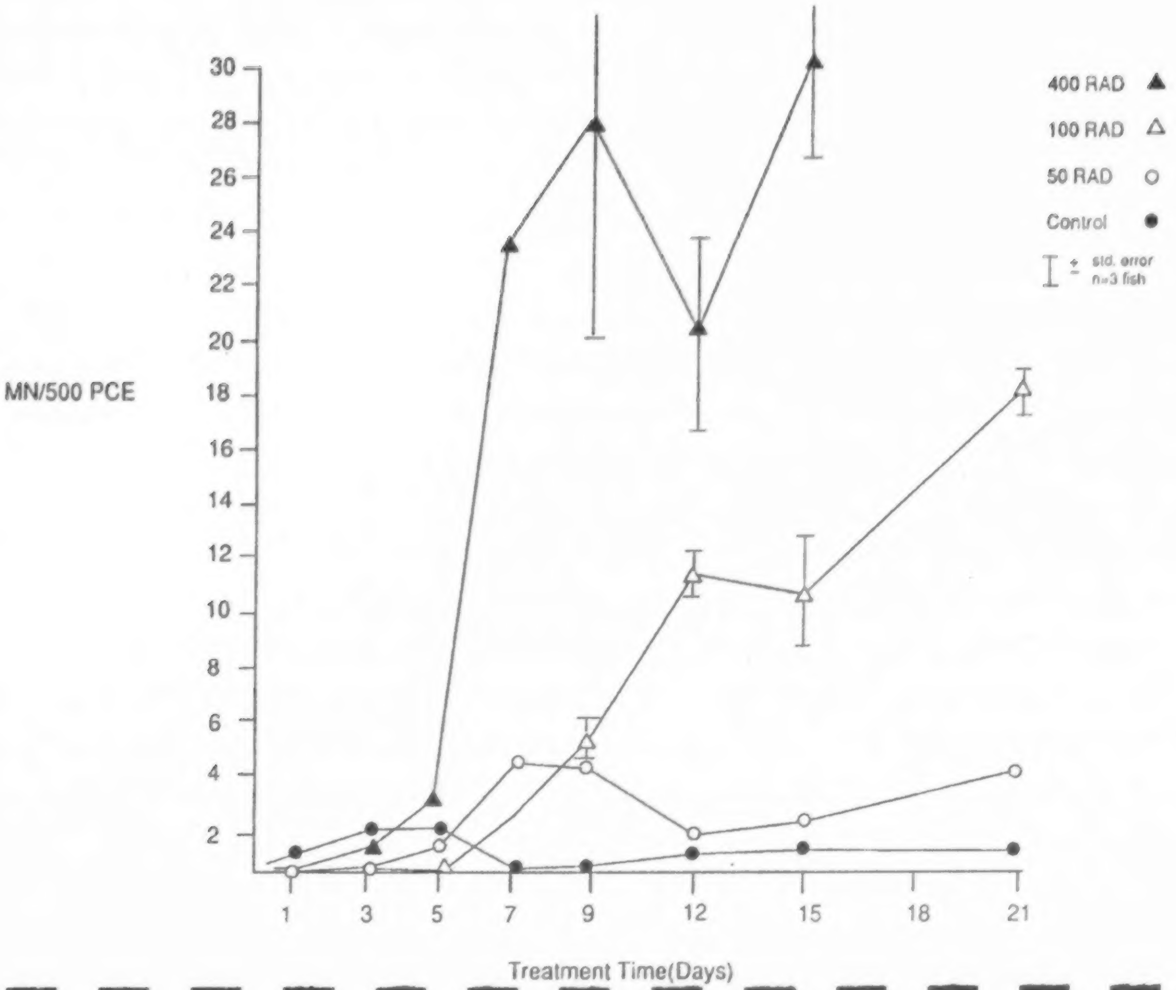


Figure 2

Micronuclei in PCE's and NCE's of Two Inch Fish (100 RAD/2 Days)

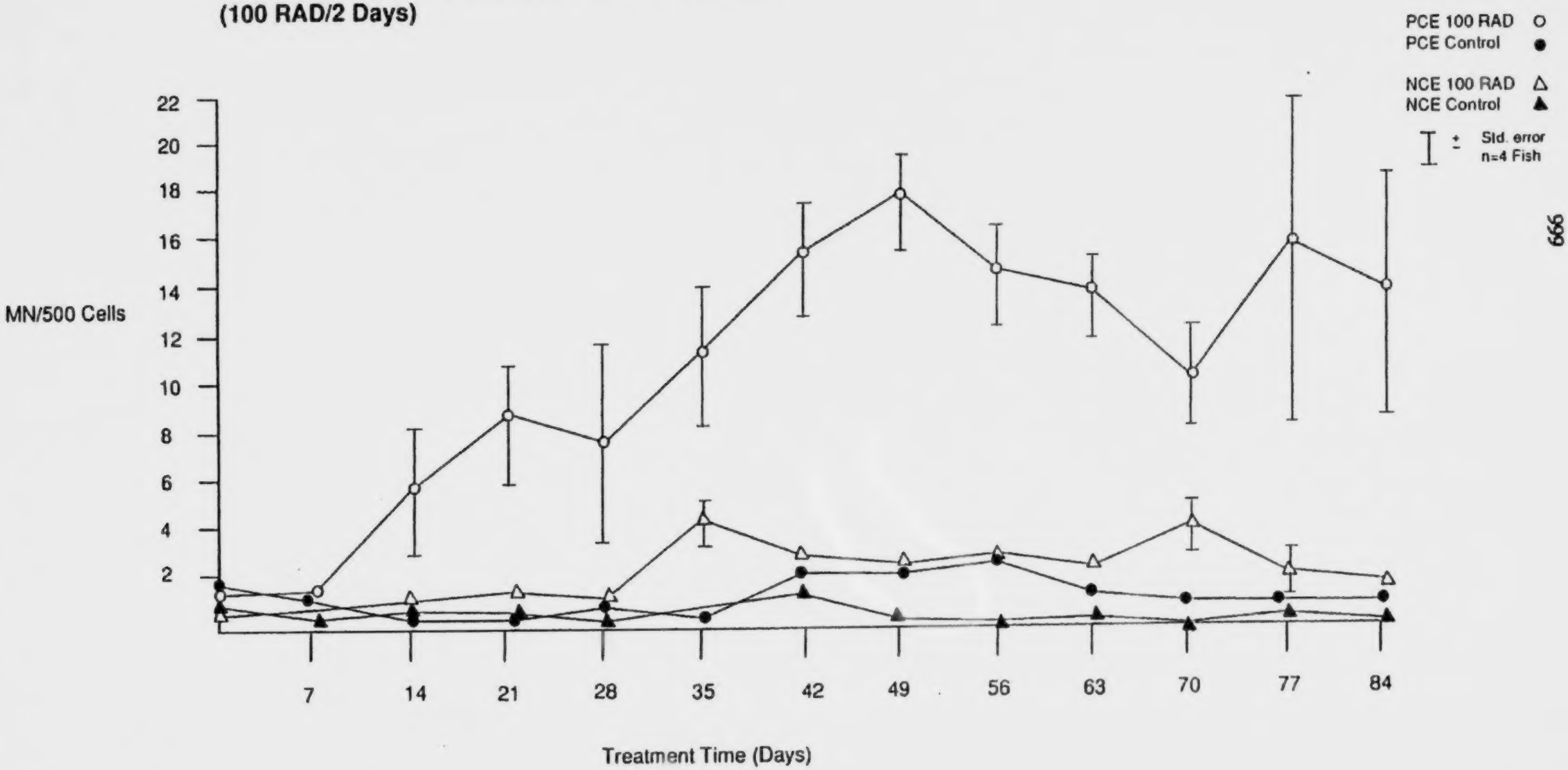


Figure 3

Two Inch Fish Weights (100 RAD/2 days)

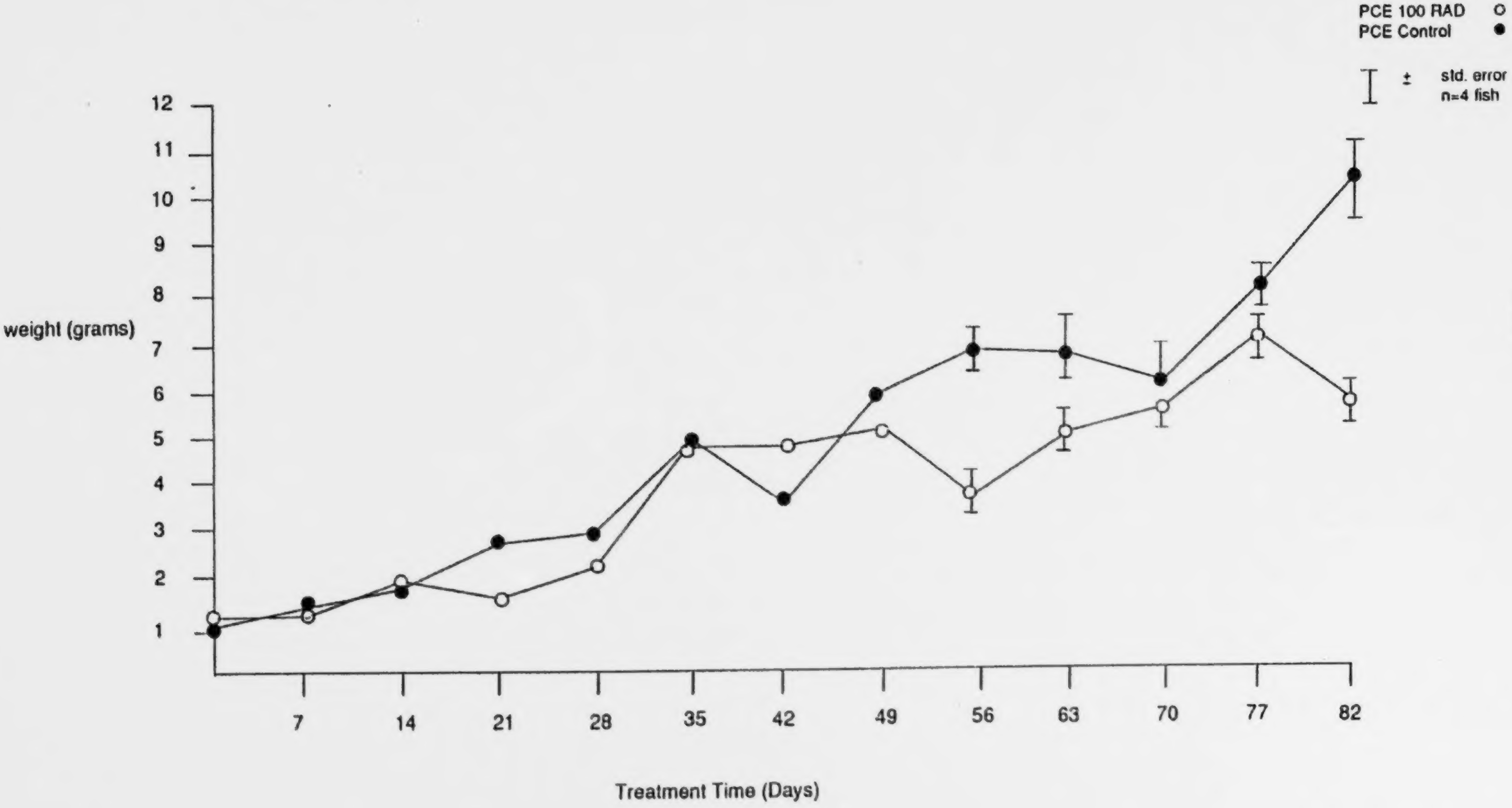


Figure 4

Micronuclei in PCE's and NCE's of Six Inch Fish (100 RAD/2 days)

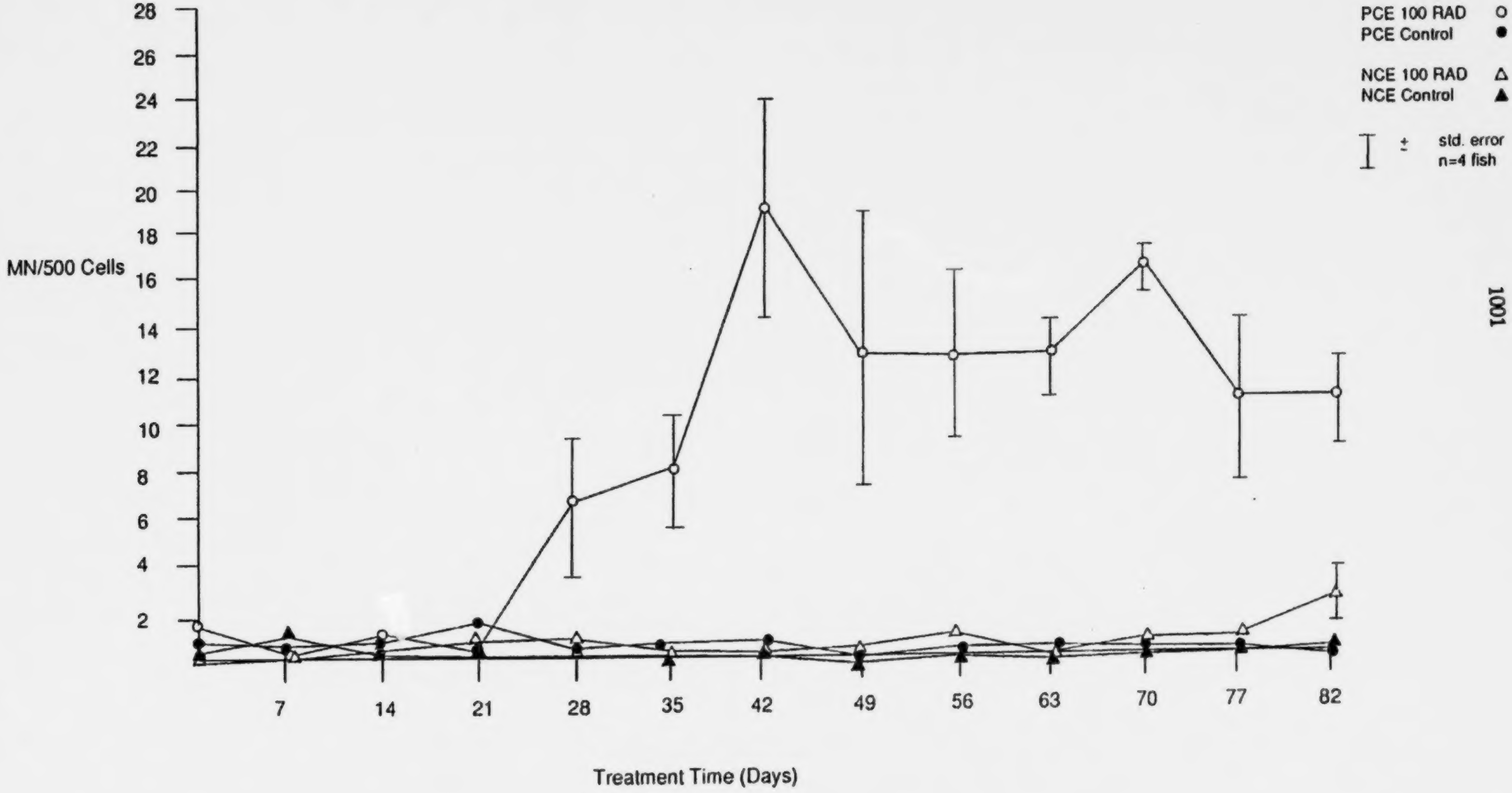
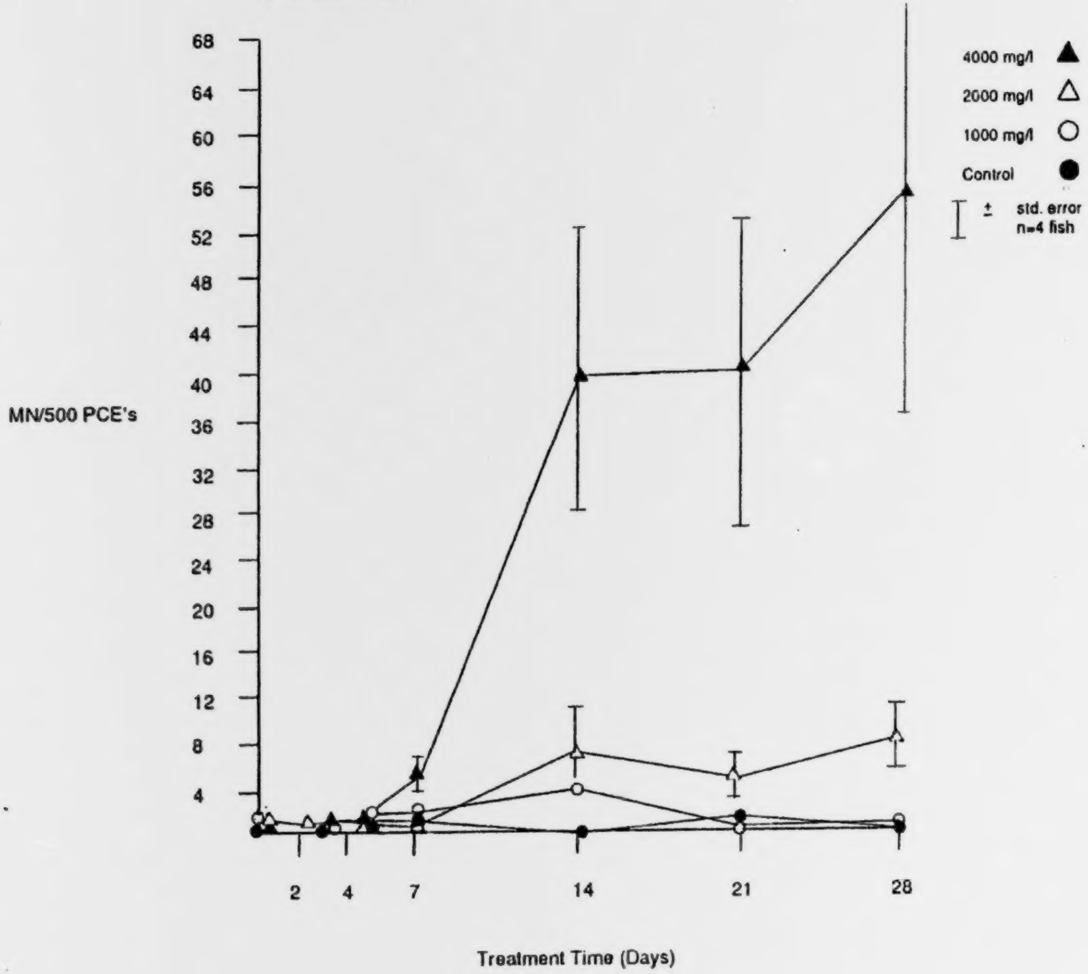


Figure 5
Micronuclei in PCE's of Two Inch Fish
(1-4 G/L TMP)



Growth effects produced by TMP were also dose-dependent (Figure 6). As with radiation exposure, growth effects and genotoxic effects were detectable at approximately the same dose level (2 g/L). At 4 g/L TMP, the NCE/PCE ratio was significantly elevated (Figure 7), suggesting a partial suppression of erythropoesis. This dose is approximately 50% of the acute LC50.

Figure 7 also suggests a marginal MN response in NCEs on day 28, at the end of the experiment. A similar pattern of response (NCEs later than PCEs) was suggested in irradiated fish (Figure 4).

Feeding with BP did not induce a MN response in small fish (Figure 8). The reasons for this are unclear. Erythropoetic activity was not suppressed, as indicated by lack of significant change in the NCE/PCE ratio. However, a growth effect was evident at the 1 g/kg dose level (Figure 9). Exposure by immersion using solvent carriers may possibly be more effective.

TABLE 1: MICRONUCLEUS RESPONSE IN PCEs OF DIFFERENT TEST ORGANISMS

			Peak MN Response (°/oo) to:	
Organism	Spontaneous MN Frequency (%)	Time to Response (days)	Radiation (100 R/day)	TMP (2 g/L)
Mouse	3	1-3	91*	45**
Frog+	3	5-9	21	-
2" Trout	2	7-14	36	16
6" Trout	2	21-28	40	-

* two day accumulation of a one day effect equal to 45.5‰(Jenssen et al 1974)

** a mouse drinking 2g/l gets 0.3g/kg/day equal to 45‰(Weber et al 1975)

+frog data from (Tomlinson et al 1988)

Based on the radiation and TMP results, we can begin to compare the sensitivity of fish, mouse and other micronucleus assays in terms of peak PCE response to equivalent doses

Figure 6
Two Inch Fish Weights
(1-4 G/L TMP)

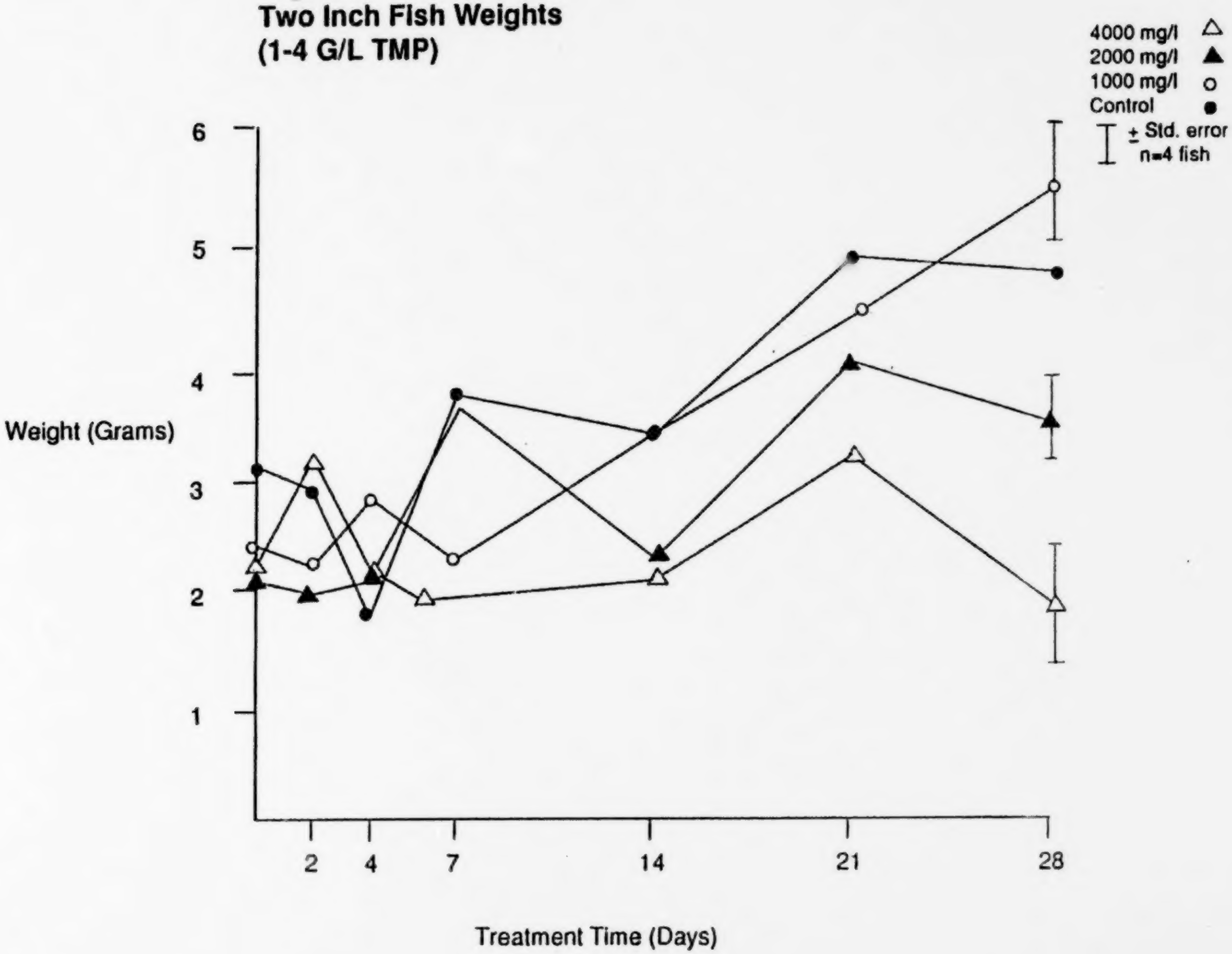


Figure 7

Micronuclei in NCE's of Two Inch Fish (4 G/L TMP)

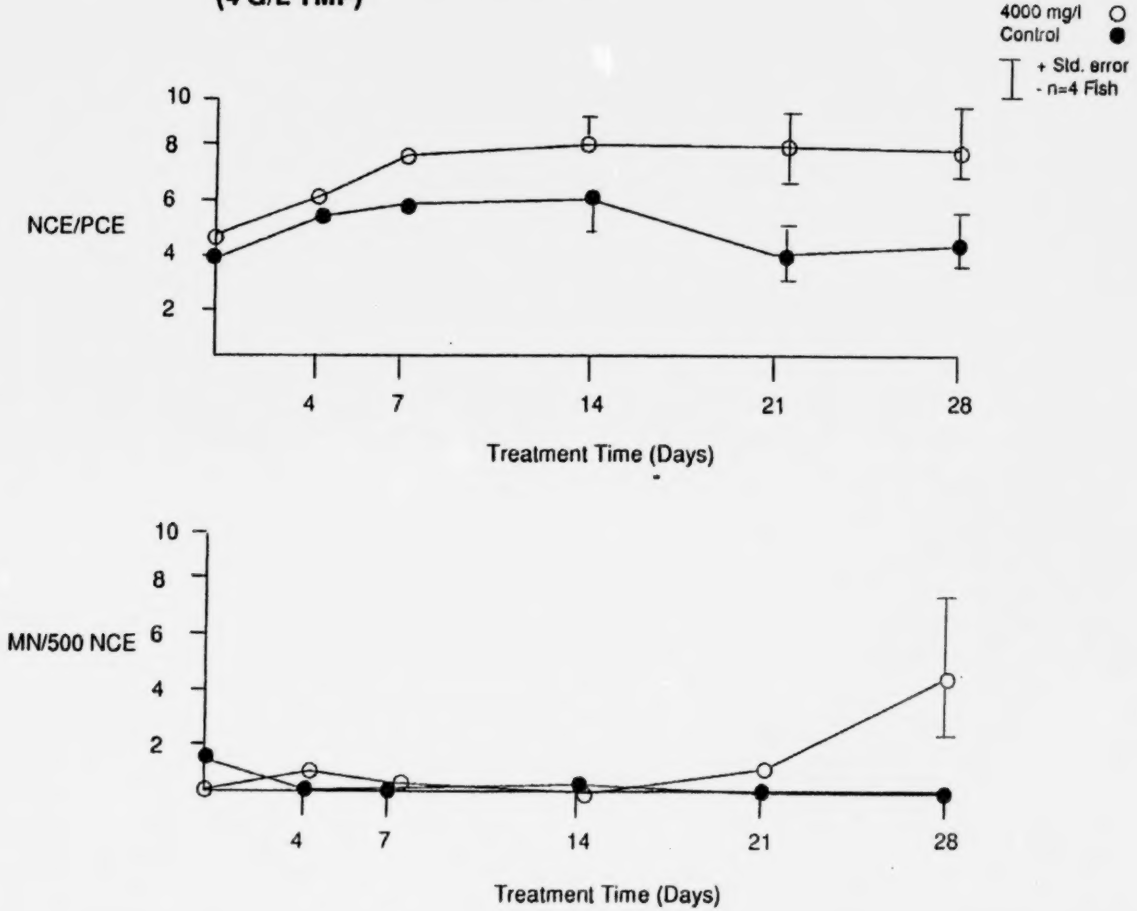


Figure 8

Micronuclei in PCE's of Two Inch Fish
(1 G/Kg B(a)P in Food)

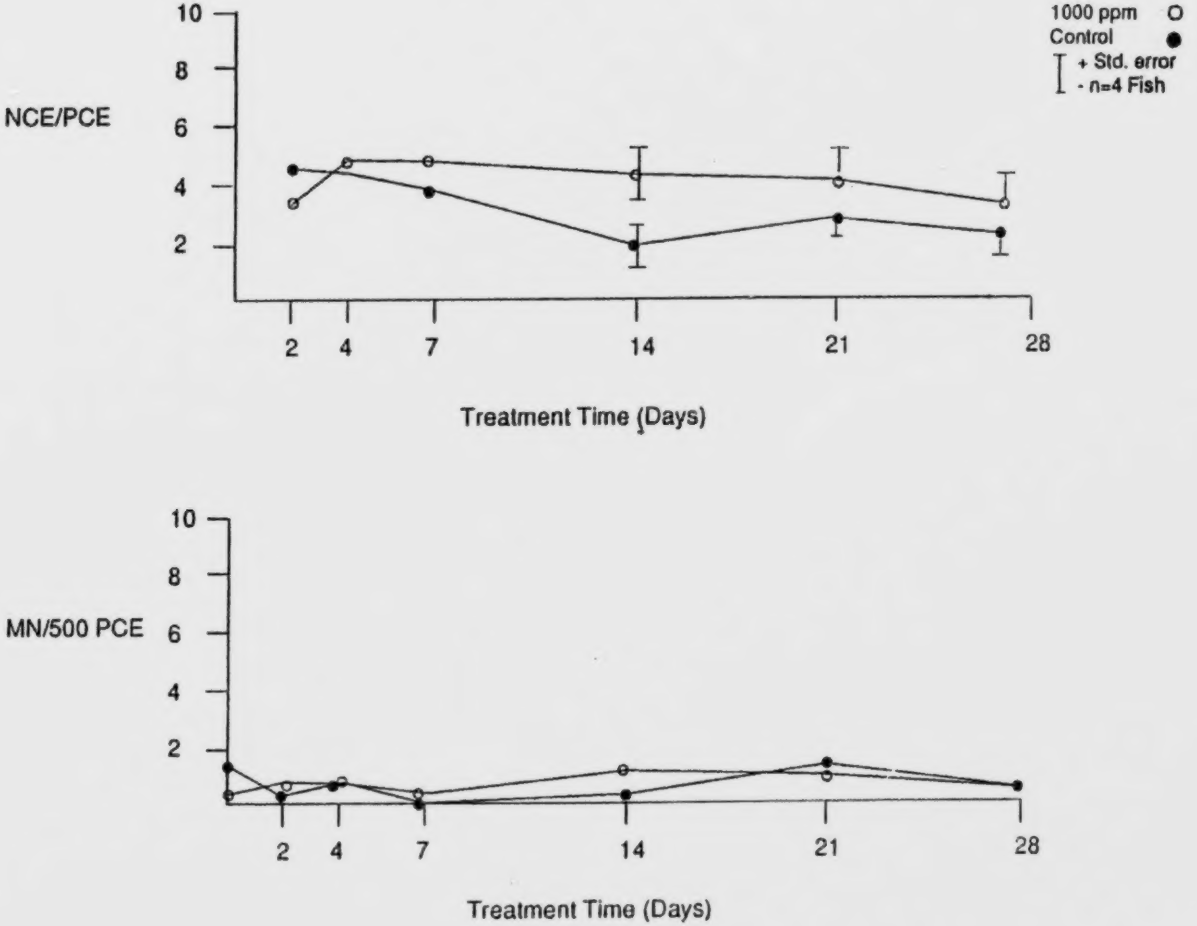
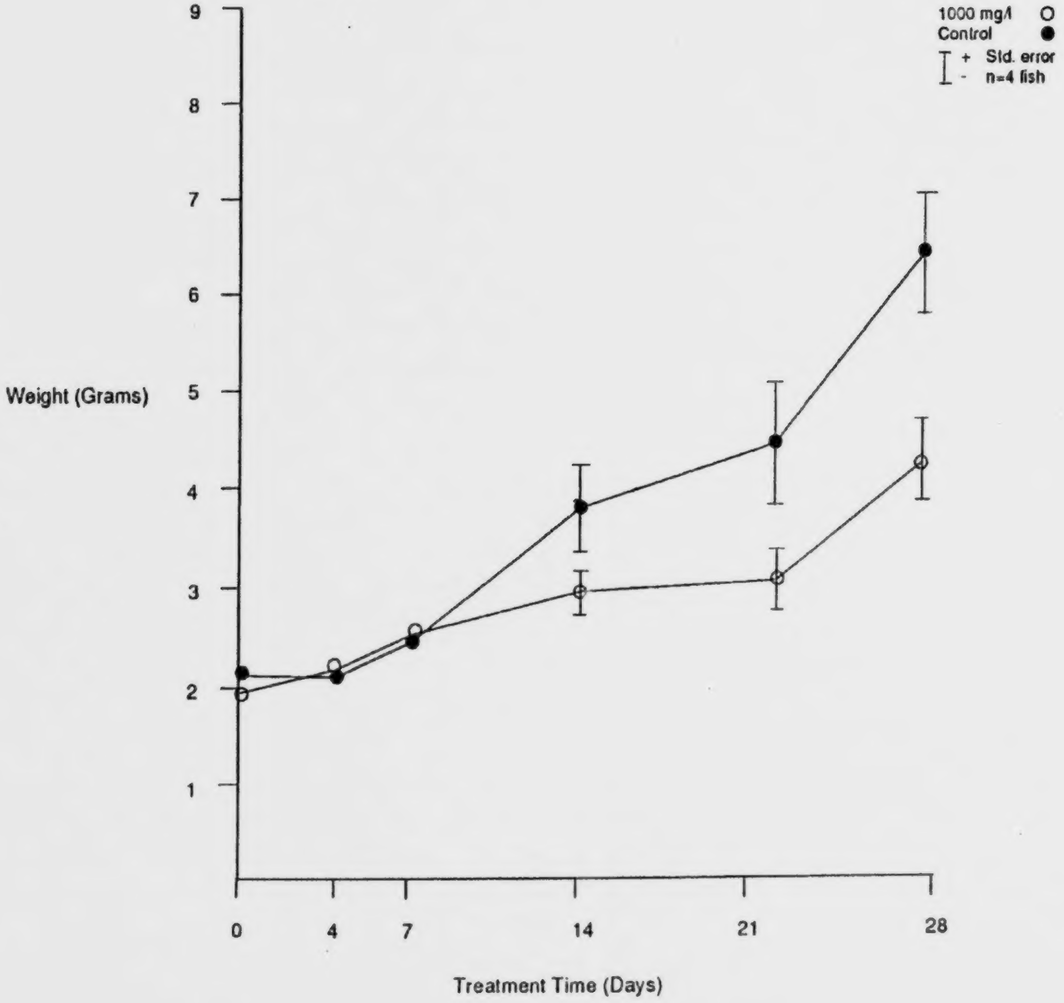


Figure 9

Two Inch Fish Weights (1 G/Kg B(a)P in Food)



(Table 1). Determination of equivalent chemical doses in aquatic and terrestrial organisms is always difficult. However, for practical purposes, we can compare the mouse response to chronic drinking of an aqueous sample, with the fish response to chronic immersion. The comparison on either radiation or TMP response suggests that the fish assay has about one-third the sensitivity of the mouse assay. The time to PCE response is greater in the fish, and response time increases with fish age/size.

The NCE response time in the fish is very long, and it is possible that there could be selection against micronucleated NCEs prior to pool saturation. This reduces the sensitivity of the NCE response and makes it impractical as an endpoint. However, the fish PCE pool seems to integrate genotoxic effects over roughly the same period of time as the NCE pool in the mouse, and may be a reasonable alternative for aquatic biomonitoring of genotoxic potential. This will ultimately depend on demonstrated sensitivity to a broad spectrum of genotoxic agents.

Other aquatic organisms have been investigated for micronucleus response; however, most of these studies have not distinguished between NCEs and PCEs. This makes direct comparison to the trout test difficult. Nevertheless, some amphibian larval systems appear promising. Van Hummelen et al. (1989) induced 4.2% MN in red blood cells (RBCs) of frog larvae *Xenopus laevis* after 14 days of EMS treatment at 100 mg/L (control response 0.2%). Both control and treatment responses were dependent on larval stage and temperature (greater in young larvae at high temperature, 26°C). They also induced 6.0% MN in RBCs after 12 days of treatment with BP in solution at 0.5 mg/L. Fernandez et al. (1989) induced 20.6% MN in RBCs of newt larvae (*Pleurodeles walti*) after 12 days of EMS treatment at 100 mg/L, and 30.4% MN after 12 days of BP treatment at 0.1 mg/L (control response 0.7%). Twelve days were shown to be sufficient for maximum response (Jaylet et al. 1986). Krauter et al. (1987) induced 1.6% MN in RBCs of frog larvae (*Rana catesbeiana*) 15 days after a single acute radiation exposure of 210 rad (control response 0.4%). Peak response occurred on days 13 to 17, but was greater in young larvae.

Fish micronucleus assays appear to be rather less sensitive than larval amphibian assays when PCEs are not distinguished. Hooftman and deRaat (1982) induced 0.4% MN in RBCs of adult mudminnows (*Umbra pygmaea*) after six weeks of EMS treatment at 40 mg/L (control response 0.01%). Time to peak response was not determined. Our results suggest that scoring in PCEs of young trout may increase sensitivity to compare favourably with amphibian larval RBC endpoints (*P. walti* excepted). Young trout have the added advantage that they are readily available and widely used in routine aquatic toxicity testing.

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SUBCELLULAR INDICES AS POTENTIAL ENVIRONMENTAL MONITORS. B.E. Imber, A. Sutherland, and C.J. Myers, C.B. Research International Corporation. Sidney, British Columbia, Canada (604) 655-1944; and C.J.P. McKean, Water Management Branch, British Columbia Ministry of Environment, Victoria, BC, Canada.

The use of subcellular markers for the determination of environmental stress from both metals and organics is reviewed. Evaluation of mixed function oxidase as general indicators of organic stress and specific metal binding proteins for metal stress are discussed. Data obtained from trout and oysters from British Columbia are examined and the potential practical usefulness of these techniques is examined. Examples of MFO as applied to groundwater runoff and the induction of metal binding proteins in fish exposed to mine tailings are presented. In addition, the relationships between species and biological effect are investigated.

7-ETHOXYRESORUFIN DEETHYLASE (EROD) INDUCTION IN CHINOOK EXPOSED TO REFINERY STORMWATER RUNOFF. C.J. Myers, B. Imber, C.B. Research International Corporation. Sidney, BC Canada (604) 655-1944; and C.J.P McKean, Water Management Branch, British Columbia Ministry of Environment, Victoria, BC.

The demand for assessment of the potential health risks associated with exposure to environmental contaminants has led to the development of a number of analytical methods. CBR International is evaluating methods that measure, in fish, biochemical parameters induced in response to toxic organics. A feasibility study to assess the use of Mixed Function Oxidases (MFO) as indicators of toxic stress in fish has been performed. This study involved monitoring EROD activity in Chinook exposed to seawater containing 10% and 30% stormwater. Elevated EROD activities were observed in both the 10% and 30% stormwater-treated fish and a dose-related EROD response was apparent. Relationships between EROD activities, Microtox data, gill histological changes, and water quality will be discussed.

WORKSHOP

Sediment Quality Guidelines

Chair: M. Taylor

Summary: D. MacDonald

THE EQUILIBRIUM PARTITIONING APPROACH FOR ASSESSING SEDIMENT QUALITY.

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Equilibrium Partitioning (EqP) sediment quality criteria (SQC) are the EPA's best recommendation of the concentration of a substance in sediment that will not unacceptably affect benthic organisms or their uses. EqP is being developed for the purpose of deriving National SQC for assessment and remediation activities. Early efforts considered a variety of methods to assess sediment quality. Recent review by EPA's Science Advisory Board of a variety of approaches that could be used to assess sediment quality has been completed with particular emphasis on EqP and AET methods. Although EqP SQC are similar to existing water quality criteria the application sediment criteria may vary significantly from the way water quality criteria are applied. It is not anticipated that the primary role of sediment criteria to be used as mandatory cleanup levels. Sediment criteria can be used as a means for predicting or identifying the degree and spatial extent of contaminated areas such that more informed regulatory decisions can be made.

SPIKED SEDIMENT BIOASSAYS AS AN APPROACH TO THE DEVELOPMENT OF SEDIMENT QUALITY GUIDELINES. J.Q. Word and J.A. Ward. Battelle Marine Sciences Laboratory, 439 West Sequim Bay Road, Sequim, Washington (206) 683-4151.

Toxicological tests of marine sediments contaminated by PAHs showed toxicity changes relative to sediment stability and/or disturbance. The amphipod *Rhepoxynius abronius* was exposed to sediment undergoing no stability, stability over 10 to 35 days prior to testing, and 35-day stability followed by disturbance and immediate testing. Test results showed acute toxicity decreased with longer periods of stability, and stability followed by disturbance produced an acute toxicity which was similar to the non-stabilized result. One possible reason for these responses is that contaminants may become less available over time due to their decreasing concentration in pore-water. Previous work demonstrated that radio-labelled DDT spiked into sediment showed that pore-water concentrations of DDT reduced and gradually stabilized over a period of 30 to 40 days, and that the available toxic portions were related directly to pore-water concentrations. These tests indicate that sediment stability/disturbance is related to apparent contaminant availability, and that results of acute toxicity tests involving *R. abronius* must take this into consideration when sediment quality guidelines are developed.

DEVELOPMENT AND APPLICATIONS OF SEDIMENT QUALITY CRITERIA USING THE APPARENT EFFECTS THRESHOLD APPROACH.

R.G. Albright and J.M. Malek. U.S. Environmental Protection Agency, Region 10, 1200 Sixth Avenue, Seattle, Washington, USA (206) 442-8514.

Development of sediment quality criteria is important to provide regulatory agencies and resource managers the basis of remediating and managing existing pollution problems, as well as preventing future contamination. The U.S. Environmental Protection Agency Region 10 (EPA) and the Washington Department of Ecology (Ecology) have used the apparent effects threshold (AET) approach to develop proposed sediment quality criteria for Puget Sound. Ecology has incorporated the proposed criteria into their draft sediment management standards. The AET approach bases sediment quality criteria upon observed relationships between levels of sediment contamination and associated biological effects. Typically, development of sediment quality criteria using this approach relies upon a substantial database; however, once the database is developed, it can generate criteria values for all contaminants associated with adverse biological effects. As the database is expanded, numbers may become more broadly applicable. The approach has already proved useful for making management decisions in relation to dredging and dredged materials disposal in Puget Sound, classifying marine sediments as clean or contaminated, guiding Superfund cleanup decisions, and targeting point and non-point source control efforts.

SEDIMENT QUALITY TRIAD APPROACH.

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The determination of pollution-induced contamination by chemical substances known to be toxic in some circumstances is reasonably straight-forward. However, estimating the potential consequences, i.e., whether the particular types and levels of that contamination are actually degrading the environment, is not often as clear. The Sediment Quality Triad approach consists of collecting synoptic measures of sediment contamination: a) the concentrations of selected chemical substances, b) sediment toxicity using bioassays, and c) a measure of possible *in situ* biological effects, most commonly benthic infaunal community structure. The major advantage of the Triad approach is that each component of the Triad complements the limitations of the other two to provide a means of determining areas where pollution-induced degradation has occurred. This approach has been applied to data from a number of locations to identify degraded areas.

SEDIMENT QUALITY GUIDELINES DEVELOPED FOR THE NATIONAL STATUS AND TRENDS PROGRAM

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The concentrations of selected potentially toxic chemicals in marine and estuarine sediments have been quantified annually by NOAA in the National Status and Trends (NS&T) Program since 1984. Sediments from about 200 sites nationwide have been sampled and analyzed for a variety of trace metals, petroleum hydrocarbons, and synthetic organic compounds. The chemical concentrations have been compared among sampling sites and among years at many of the sites. These data have been useful in characterizing the chemical conditions at sampling sites (NOAA, 1987, 1988) and in determining whether or not conditions are changing over time. In selected geographic areas measures of biological effects have been performed to accompany the chemical analyses and used to indicate the significance of the sediment contamination. However, biological measures of the effects or potential for effects of these mixtures of chemicals have not been determined at the majority of the sites.

Informal guidelines were developed for use the the NS&T Program to put the data from new measurement into perspective. They were developed by employing a preponderance of evidence assembled from a variety of approaches and from data gathered in many geographic areas. These guidelines were used to rank and prioritize the NS&T Program sites with regard to the relative potential for contaminant-induced effects. These guidelines were not intended for use in regulatory decisions or any other similar applications.

The data that were assembled from 85 reports, the full explanation of the approach used and the guidelines for all NS&T Program analytes were reported by Long and Morgan (1990), available from the author. A brief synopsis of that report is presented in this extended summary.

OVERALL APPROACH

The approach involved assemblying and reviewing currently available information in which estimates of the sediment concentrations of chemicals associated with adverse biological effects have been determined or could be derived; and determining the apparent ranges in concentrations of individual chemicals in which effects were often observed, based upon a preponderance of evidence. About 150 reports were reviewed. Of those, the data were used from about 85 reports in which either (1) effects-based sediment quality values were reported, or (2) in which matching chemistry and biological effects data were listed, followed by an evaluation of the co-occurrence of chemical concentrations with measures of effects. These reports embraced controlled laboratory studies of effects of sediments spiked with individual chemicals (i.e., the spiked-sediment bioassay approach), calculations of unacceptable concentrations based upon theoretical equilibrium partitioning principles, and evaluations of data from field studies in which matching chemical and biological measures were performed on subsamples of sediments.

Chapman (1989) reviewed and compared the approaches currently being pursued to develop sediment quality values, but did not compare the concentrations resulting from those approaches. That report should be consulted for more information on each of the respective approaches. One approach not described by Chapman (1989) that was used extensively in the development of the NS&T Program guidelines was a co-occurrence approach. This method uses matching biological and chemical data collected in field studies. It involves calculation of statistics of central tendency (*i.e.*, means, standard deviations, maxima, minima) in chemical concentrations associated with matching samples determined to have high, intermediate, and low indications of effects. Data were assembled from many studies conducted in many different geographic locations in North America and elsewhere. Both freshwater and marine data were used.

The data from the 85 reports were assembled and listed for each of the NS&T Program analytes according to the type of approach or type of test that was used, and, then, subjected to a screening step. If matching chemical and biological data from field studies showed no concordance, the data were not given further consideration. If no gradient (generally, less than a two-fold difference) in chemical concentrations was reported between samples that indicated adverse effects and those that did not indicate effects, the data for that particular chemical also were not given further consideration. If no definitive Apparent Effects Threshold (AET) concentration could be determined, the "greater-than" value reported was excluded from further consideration. The screening step was not performed to force consensus where none existed. The data that remained following this screening step were from studies in which effects were either predicted or observed in association with increasing concentrations of the respective analyte. Then, they were sorted in ascending order.

Next, two guidelines were determined from these remaining, sorted data for each chemical: an ER-L (Effects Range-Low), a concentration at the low end of the range in which effects had been observed; and an ER-M (Effects Range-Median), a concentration approximately midway in the range of reported values associated with biological effects. These two values were determined using a method similar to that used by Klapow and Lewis (1979) in establishing marine water quality standards for the State of California. For each chemical of interest, they assembled available data from spiked-water bioassays, examined the distribution of the reported LC50 values, and determined the lower 10- and 50-percentile concentrations among the ranges of values. In the present document, the ER-L values were concentrations equivalent to the lower 10 percentile of the screened, sorted data, and indicated the low end of the range of concentrations in which effects were observed or predicted. They were used in the document as the concentrations above which adverse effects may begin or are predicted among sensitive life stages and/or species or as determined in sublethal tests. The ER-M values for the chemicals were the concentrations equivalent to the 50 percentile point in the screened, sorted data. They were used in the document as the concentrations above which effects were frequently or always observed or predicted among most species.

In addition to the objectively determined ER-L and ER-M values, overall apparent effects thresholds were subjectively identified for some chemicals. These thresholds were the concentrations above which effects usually or always occurred in association with increasing concentrations of the chemical. They were determined independently of the ER-L and ER-M values by visually examining the sorted, ascending data tables. They are not to be confused with the AET values reported for Puget Sound, San Francisco Bay, and Mississippi Sound. They were identified as an aid in evaluating the accuracy of the ER-L and ER-M values and were not used in ranking the NS&T Program sites.

Data compilation and analysis was as inclusive as possible and no weighting was given to data derived from one approach or another. As Klapow and Lewis (1979) pointed out, the use of the inclusive approach and the calculation of percentiles of the data help eliminate the undue influence of a single (possibly outlier) data point upon the establishment of consensus ranges in concentrations associated with effects. In the present evaluation, the assumption was made that patterns established between effects and chemical concentrations would be more credible if based upon data from several sediment quality criteria than if based upon data from only one approach or experiment.

The relative degrees of confidence in the accuracy of the ER-L and ER-M values were subjectively judged for each analyte. Values for which we had relatively high confidence were those that were supported by clusters of data with similar concentrations, by data derived from more than one approach, by a data set that included more than results from the use of the co-occurrence approach, by data derived from multiple geographic areas, and for which the overall apparent effects threshold was similar to or within the range of the ER-L and ER-M values. Values for which we had relatively low confidence were those that were supported by data with either a small cluster or no cluster of similar concentrations, by data derived from only one approach and/or from one geographic area, results derived only from the co-occurrence approach, and for which the overall apparent effects threshold was dissimilar to or outside the range of the ER-L and ER-M values.

SUMMARY OF GUIDELINE CONCENTRATIONS

The ER-L and ER-M concentrations for each chemical or chemical group determined by Long and Morgan (1990) are listed in Table 1. These guidelines were used to evaluate the potential for toxic effects in sediments at the NS&T Program sites and to rank the sites.

The available data for some chemicals indicate agreements among the various approaches and the various data sets that were evaluated. For example, there is a relatively large amount of data available for cadmium generated from a variety of approaches and studies. The Puget Sound AET concentrations range from 5.1 ppm to 9.6 ppm; the 10-d LC50 concentrations from many spiked-sediment bioassays with amphipods range from 5.6 to 11.5 ppm; and significant toxicity to amphipods and reduced echinoderm abundance in Southern California sediments occurred in samples with mean cadmium concentrations of 5.3 and 6.2 ppm, respectively. Effects were not observed in sediments with cadmium concentrations of less than about 4 ppm. With some exceptions, biological effects were usually observed in association with cadmium concentrations of 5 ppm or greater. The preponderance of evidence from these data suggest that effects are likely or expected as cadmium concentrations in sediments reach about 5 ppm. Also, the effect of adding or deleting data upon the ER-L and ER-M values for cadmium would likely be relatively small.

For some other chemicals, there was less agreement among the data from various approaches and the degree of confidence in the accuracy of the resulting ER-L and ER-M values was relatively low. For example, the Puget Sound AET concentrations for chromium are 260 and 270 ppm, whereas effects were observed elsewhere in association with mean concentrations as low as 61 ppm and as high as 1646 ppm. Many of the biological measures of effects were not in concordance with chromium concentrations, suggesting that chromium had a minimal role or no role in causation. In another example, the Screening Level Concentrations for total PCBs range from 2.9 ppb to 42.6 ppb based upon a relatively large amount of data; whereas, the Puget Sound AET concentrations range from 130 ppb to 3100 ppb, the San Francisco Bay AET range from 54 to 260 ppb, the chronic marine threshold predicted by Equilibrium Partitioning methods is 280 ppb, and the LC50 from a spiked sediment bioassay performed with amphipods is 10800 ppb. The effect of adding or deleting data upon the ER-L or ER-M values could be significant for some of the chemicals for which there is little consistency or clustering in the data. Obviously, for many chemicals there is yet much to be learned as regards the chemical concentrations in sediments that cause biological effects.

Overall, the degree of confidence in the accuracy of the ER-L and ER-M values should be considered as moderate for the metals group and PCBs and low for the pesticide and PAH groups. Much more data are needed to support or refute the ER-L and ER-M values for all groups and for individual analytes within the groups. Since no data were available for tropical areas, for most of the southeastern USA, or for subarctic areas, the applicability of these guidelines to those areas is uncertain.

Also included in Table 1 is a summary of the subjectively determined, overall apparent effects thresholds for each chemical; the concentrations equal to and above which biological effects were usually or always observed. The ER-L and ER-M values were established objectively with *a priori* selection criteria, i.e., the lower 10 percentiles and 50 percentiles of the available data. They were not established following review and evaluation of the data for each chemical. However, following a review of the available data for each chemical, apparent effects thresholds were often observed and noted. These thresholds were established with a subjective approach. Therefore, they were identified and listed as evidence to support the strength of the ER-L and ER-M values and as hypotheses to be evaluated with additional data. They were not used to rank the NS&T Program sites. For several chemical analytes (i.e., chromium, total DDT, dieldrin), there was no apparent effects threshold. No effects were observed in some studies where high concentrations of these chemicals occurred. For nickel and many of the pesticides and aromatic hydrocarbons, there were insufficient data to determine a threshold, noted as not sufficient data (NSD) in Table 1. For many of the analytes, e.g., mercury, there were inconsistent data at concentrations above the apparent effects thresholds, i.e., data from some studies indicated no effects at relatively high concentrations of the analyte. There was a very distinct threshold for some chemicals, i.e., arsenic, cadmium, copper, lead, mercury, zinc, PCBs, anthracene, and some other aromatic hydrocarbons. The apparent effects thresholds for most of the trace metals, PCBs, DDT, and some of the aromatic hydrocarbons were very similar to the respective ER-M values or within the ER-L/ ER-M range. However, the apparent threshold was outside the ER-L/ER-M range for antimony and lead.

SUGGESTED USES OF THE GUIDELINES

Since the report by Long and Morgan (1990) was published, the ERL and ERM guidelines have been used by others in applications other than the evaluation of the NS&T Program data. Some questions have arisen as a result of others using the guidelines. Clarification of the suggested uses of the data reported by Long and Morgan (1990) are, perhaps, in order.

First, instead of using the ERL and ERM values alone, it is suggested that investigators also compare their new data with the ascending, sorted data presented in Appendix B of the report. Determine how the new data compare with observations or predictions made by others. Determine if the concentrations in the new data equal or exceed the concentrations previously associated with toxic effects or not, and note what types of effects were observed and the approach that was used. For example, assume that the chemical analysis of a sample indicated that it contained 4 ppm fluoranthene. Examination of the data in Appendix B-25 of Long and Morgan (1990) indicates that this concentration exceeds the ERM value (3.6 ppm), the overall apparent effects threshold (1 ppm), an LC50 from a spiked sediment bioassay, the mean concentration associated with effects in Commencement Bay and San Francisco Bay, AET concentrations determined with a variety of different biological tests, marine screening level concentrations, and the toxic concentrations predicted with the equibilibrium partitioning approach. With this weight of evidence, one could assume that the sample with 4 ppm likely would present a toxicological problem.

Second, the investigator should examine the data tables in the report to determine how applicable the data may be to the evaluation of his/her new measurements. That is, new data collected from the southeastern portion of the USA, for example, may not be comparable with those assembled in the tables. Also, the investigator may have low confidence in certain approaches or particular data sets that were used by Long and Morgan (1990), and may elect to ignore or reject some of those data.

Third, it should not be assumed that the data used in the document necessarily reflect cause/effect relationships. The approaches that were reported often reflected merely the association between the concentrations of chemicals and the observations of toxic effects in the same samples. No cause/effect relationship was necessarily implied.

Long and Morgan (1990) accepted and used data from many different studies that undoubtedly relied upon different analytical methods. Sediment bioassays were performed with many different species and included different toxicological end-points. Extraction and analytical methods for chemical analyses differed among the studies. The models used to predict equilibrium-partitioning constants improved and changed iteratively as new information was acquired. Some biogeographic areas are not represented by any data. Freshwater and marine data were merged and treated as if they were comparable, whereas some toxicants may not be equivalent in bioavailability in the two regimes. No attempt was made to normalize the chemical data to total organic carbon, grain size, acid volatile sulfides or any other parameters. Therefore, caution is advised in using the ERL and ERM guidelines unequivocally as absolutes. They should be used as informal guidelines and should be used along with the evidence listed in the ascending, sorted tables.

Finally, it should be understood that there is a need for additional information for all of the analytes. There seems to be very little consensus among the data for some chemicals. The reasons behind this lack of agreement should be explored and determined. On the other hand, relatively good agreement is apparent for some chemicals - additional data should be acquired to further test (and verify) the effects range despite the occurrence of a consensus.

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AN APPROACH TO THE DEVELOPMENT OF SEDIMENT QUALITY OBJECTIVES FOR BURRARD INLET

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ABSTRACT

Sediment quality objectives have been proposed for Burrard Inlet by the B.C. Ministry of Environment. The general principle followed in developing these was, if possible, to set the objective below the lowest measured AET from Puget Sound, while using data for B.C. reference sites to reflect local conditions. Finally, data for the Puget Sound reference site were used as a back-up to maintain perspective. The objectives for PAHs were usually 10 % of the lowest AET, while those for metals were always less than the AET, except those for chromium and nickel.

INTRODUCTION

A recent Environment Canada study has highlighted that English sole from Burrard Inlet have high prevalences of idiopathic liver lesions (Goyette et al. 1988). Myers et al. (1987) have illustrated a link by association between histopathological conditions in demersal fish and xenobiotic chemicals in sediments and sea water.

Burrard Inlet is a major marine harbour located in the Greater Vancouver Regional District (GVRD) of British Columbia, Canada. Burrard Inlet encompasses three main bodies of water (Figure 1). The eastern basin of the inlet starts at Port Moody, and continues westward for about 13 km to Second Narrows. The major volume of water in the eastern portion of the inlet is in a fjord called Indian Arm, which extends north about 22 km.

Vancouver Harbour is the body of water between First and Second Narrows, and is about 2.5 km wide and 8 km long. Second Narrows is about 600 m wide, while the width of First Narrows is 450 m. Tidal action, and the constrictions at both First and Second narrows, create significant flushing of the inlet, with peak velocities at each of the narrows reaching 6 m/s.

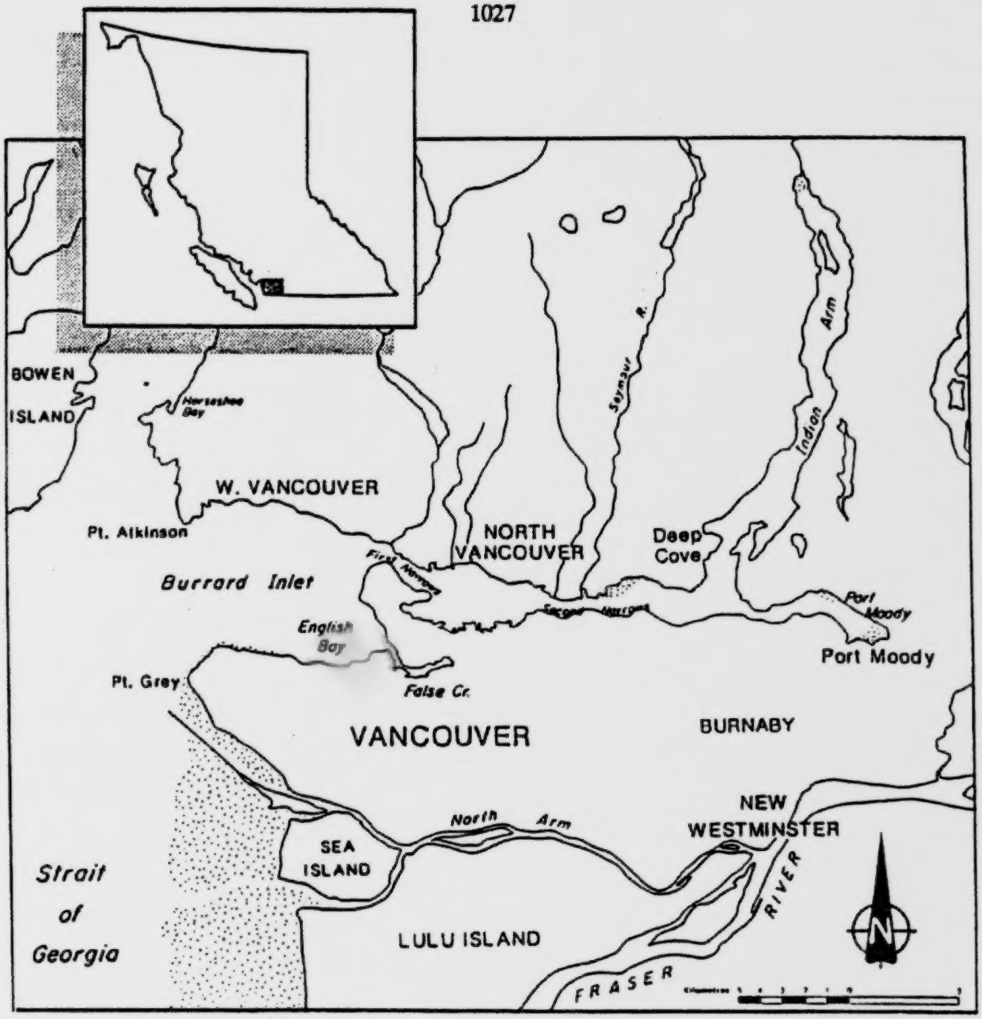
BOWEN
ISLAND
Horseshoe Bay
W. VANCOUVER
Pt. Atkinson
Burrard Inlet
First Narrows
NORTH VANCOUVER
Seymour R.
Indian Arm
Deep Cove
Second Narrows
Port Moody
Port Moody
English Bay
Pt. Grey
False Cr.
VANCOUVER
BURNABY
NEW WESTMINSTER
North Arm
SEA ISLAND
Strait of Georgia
LULU ISLAND
FRASER RIVER
N

Figure 1. The Burrard Inlet Study Area

West from First Narrows the inlet opens to the Strait of Georgia, and has a width of about six kilometres from Point Grey to Point Atkinson. Ships often anchor in this area awaiting a terminal berth.

Burrard Inlet is the major port for Western Canada, with port facilities being concentrated between First and Second Narrows, and in Port Moody Arm. Port facilities include passenger terminals, bulk loading terminals for grain, ores,and other materials, rail and barge trans-shipment facilities, general cargo terminals, and commercial and recreational marinas (Nijman and Swain 1990).

The B.C. Ministry of Environment (MoE) is preparing water quality objectives for priority water basins of British Columbia, on a site-specific basis (MoE 1986). The objective can be a physical, chemical, or biological characteristic of water, biota, or sediment, which will protect the most sensitive designated water use for a particular location (MoE 1986). The work described hereafter is that which was undertaken to formulate water quality objectives for the bed sediments in Burrard Inlet.

Water quality objectives are aimed at protecting the most sensitive water use with due regard to ambient water quality, aquatic life, waste discharges, and socio-economic factors (MoE 1986). Water quality objectives are based on approved or working water quality criteria which are characteristics of water, biota, or sediment that should not be exceeded to prevent detrimental impacts to the water use (MoE 1986). Water quality objectives have no legal standing but are used as a reference point to determine the state of the environment. Water quality objectives are used also in preparing limits for Waste Management Permits, which do have legal standing.

OCEANOGRAPHY OF BURRARD INLET

The following description of the oceanographic features of Burrard Inlet is extracted largely from Department of Environment (DOE) (1971). Burrard Inlet is relatively shallow between First Narrows and its head at Port Moody, with a mean depth of 21 m and a maximum depth of 66 m in Vancouver Harbour. The shorelines are of moderate slope. Port Moody Arm is a relatively shallow arm of Burrard Inlet, with little direct fresh water inflow. Its oceanography is influenced mostly by the rest of Burrard Inlet and Indian Arm. In contrast, Indian Arm is a typical deep inlet , or fjord, with a mean depth of about 120 m, a maximum depth of 218 m, a sill at the entrance, and steep mountain walls.

Fresh water drainage to Burrard Inlet includes numerous rivers and streams, a power plant that diverts water from the Coquitlam Lake drainage, and runoff from surrounding urban areas. Local runoff is heaviest from autumn through spring. The Fraser River is also a source of fresh water to Burrard Inlet, contributing peak flows in the early summer.

Tidal currents contribute the most towards mixing and flushing, with wind-stirring, evaporation, and tidal waves also being factors. The mean tide is 3 m although the maximum can reach 4.9 m. Tides are mixed, with both diurnal and semi-diurnal oscillations. The estuarine circulation in Burrard Inlet, including the seaward movement of fresh water and entrained salt water together with compensating up-inlet flow of deep water from the Strait of Georgia, is enhanced by a predominantly easterly wind which drives surface water towards the mouth of Burrard Inlet.

The salinity of surface waters is lower than that of the deeper water due to freshwater at the surface. Whereas the salinity of deeper water is usually 29 to 30 ^{o}/oo, surface salinity can vary from 20 to 25 ^{o}/oo during the winter local runoff period to less than 10 ^{o}/oo during the summer when the Fraser River is the major dilution factor. The most saline surface waters in Burrard Inlet occur between the First and Second narrows due to the turbulent mixing associated with the estuarine and tidal flows through the shallow constrictions in these areas.

The temperature of the surface water in Burrard Inlet is generally higher than the deep water in the summer, with a maximum of 20 ^{o}C. This is reversed in the winter when surface water temperatures can be as low as 5 ^{o}C. Deep water temperatures are more stable, with one-third the temperature range of surface water. An isothermal condition can result from vertical mixing during very cold winters. The presence of both First and Second narrows can result in lower surface water temperatures during the summer and higher temperatures during the winter than would be found in a simpler estuarine environment.

WATER USES IN BURRARD INLET

Burrard Inlet is used for shipping and both primary and secondary contact recreation. Marinas, yacht clubs, and other boating facilities are common throughout much of Burrard Inlet. Besides power and sail boats, there are two popular canoe/kayak routes .

The harbour supports a variety of epibenthic invertebrates and demersal fish species (Goyette and Thomas 1987). In fact, catches of the major commercial and recreational species found in Vancouver Harbour, such as English sole, Dungeness crab, and pandalid shrimp, were higher than catches from other inlets on the

B.C. coast using similar equipment (Harding and Thomas 1987). Areas of Burrard Inlet such as False Creek are important rearing and protection areas for commercially important fish species (GVRD 1987).

Waterfowl are common, as is the presence of the occasional harbour seal. Burrard Inlet is on the Pacific Flyway migratory bird route linking the U.S.S.R. with South America. An example of frequented waterfowl habitats is a large waterfowl population near Stanley Park from October to May.

WASTE WATER DISCHARGES TO BURRARD INLET

The following discussion will focus on the discharges to Burrard Inlet which were deemed to have the most significant impact on water quality and resulted in water quality objectives being proposed for sediments in Burrard Inlet. A complete inventory of waste water discharges to Burrard Inlet can be found in Nijman and Swain (1990). Water quality objectives for sediment quality were established for metals, polychlorinated biphenyls (PCBs), chlorophenols, and polycyclic aromatic hydrocarbons (PAHs).

Combined sewer overflows, which are a mixture of storm water and untreated domestic sewage, occur in most areas of Burrard Inlet. Such discharges carry whatever contaminants may be contained in domestic sewage, as well as any contaminants found in storm water. Storm water discharges also occur throughout the area draining to Burrard Inlet. The storm water would contain quantities of PAHs, as well as some metals such as lead and zinc. Storm water discharges are most frequent during winter, and generally minimal from May through September.

Primary-treated sewage is discharged from the Lion's Gate Sewage Treatment Plant at First Narrows. Digested sludge has been discharged on an intermittent basis with the effluent, but this practice will be discontinued in 1991. This will eliminate the discharge of a number of metals to the Inlet. The sewage treatment plant design flow rate is 102 000 m^3/d. The average flow in 1989 was about 91 000 m^3/d (D. Littleford pers. comm.).

Vancouver Wharves is a bulk loading terminal located on the north shore of Burrard Inlet, just east from First Narrows. Waste water discharges originating from this facility relate to storm water runoff from the methanol tank farm and the ore concentrate, sulphur, potash, and phosphate storage and handling areas. Considerable impacts have been noted for metals concentrations in sediments (e.g., copper > 1 000 µg/g and zinc > 100 µg/g) at some distance from this operation (EVS Consultants 1982), as well as in tissues of resident biota. EVS Consultants concluded that there was evidence of bioaccumulation of metals in the area of the loading facilities (EVS Consultants 1982). Although works such as covering ore stock piles has been undertaken at this terminal since the early 1980's, metals concentrations in sediments near the terminal remain high.

Four oil refineries discharge contaminated storm water to Burrard Inlet. These refineries are the Shellburn Refinery (Shell Canada), the Chevron Refinery, the Petro-Canada Refinery, and the IOCO (Imperial Oil) Refinery. These contaminated discharges can introduce PAHs to the aquatic environment. In the past, process effluents were also discharged to the inlet.

THE PROCESS OF DEVELOPING SEDIMENT QUALITY OBJECTIVES

Although sediments in the different reaches of Burrard Inlet can have different chemical characteristics relative to each other, one objective for each characteristic was proposed for the entire basin. The reason for this is that it is virtually impossible to know what pre-anthropogenic concentrations in Burrard Inlet sediments might have been. This type of knowledge is required to develop realistic objectives for site-specific areas. In addition, adequate data are not available to relate sediment toxicity to particle size.

In developing sediment quality objectives for Burrard Inlet, sediment quality criteria from two sources were examined. These sources were the criteria contained in the former Ocean Dumping Control Act which are now in the Canadian Environmental Protection Act (CEPA), and criteria used for Puget Sound (Department of Ecology 1989). Data from Puget Sound for the Apparent Effects Threshold (AET) were used to estimate toxicity/concentration relationships. The AET corresponds to concentrations above which all samples were observed to have infaunal reductions relative to Puget Sound reference sediments. Reference data for sediments in relatively uncontaminated sites for Puget Sound (90th percentile) and for Loughborough Inlet in British Columbia were used to estimate pre-anthropogenic concentrations. Loughborough Inlet is located approximately 200 km northwest from Vancouver on the coast of the mainland. Finally, sediment quality data for nearby Boundary Bay and saline reaches of the Fraser River Estuary were used to obtain a perspective of realistic concentrations for Burrard Inlet. Data from what had once been deemed a control site for Burrard Inlet but later dismissed as being slightly contaminated, are referred to as a Burrard Inlet reference site.

The general principle followed was that if possible, the sediment quality objective should be:

1. Below the lowest AET for Puget Sound,
2. Greater than the lowest AET if B.C. reference sites exceed the AET, and
3. No greater than three times the mean concentrations measured at B.C. reference sites, including if possible, Boundary Bay and the Fraser River Estuary. The Burrard Inlet site is not necessarily an unaffected reference site. This factor of three times is an arbitrarily assumed factor to compensate for the normal range of data around a mean concentration.

The data for the Puget Sound reference site were used solely as a back-up to determine how realistic a proposed objective might be. The data for metals, PCBs, and pentachlorophenol are in Table 1.

TABLE 1
SEDIMENT OBJECTIVES AND DATA FOR METALS, CHLOROPHENOLS AND PCBs FOR REFERENCE SITES
(μg/g dry-weight)

Characteristic	Lowest AET	CEPA	PUGET SOUND		LOUGHBOROUGH INLET		FRASER R.		BOUNDARY BAY		BURRARD INLET		OBJECTIVE
			CRITERIA	REFERENCE	Range	x	Range	x	Range	x	Range	x	
As	85	1000	57	19	<8	<8	5.6-8.2	<6.6	1.2-11	3.6	<8-31	-	20
Cd	5.8	0.6	5.1	1.4	0.31-0.65	0.44	0.2-0.36	0.29	<0.1-1.12	0.17	0.2-0.7	0.52	1.0
Cr	27	1000	260	150	32-39	36	24-61	40	9.9-58	27	50-72	57	60
Cu	310	1000	390	58	56-72	64	16-58	38	2.8-42	11	146-220	190	100
Pb	300	0.5	450	30	21-23	22	9.6-30	17	1.2-19	5.4	37-70	48	30
Hg	0.41	0.75	0.41	0.19	0.12-0.13	0.12	0.05-0.12	0.08	0.006-0.095	0.026	0.15-0.31	0.23	0.15
Ni	28	1000	-	65	18-25	22	32-55	43	6.3-41	18	38-52	43	45
Zn	260	1000	410	110	102-135	115	51-143	90	16-110	46	142-166	150	150
PCBs	0.13	-	12 μg/gC	0.03	0.02-0.05	0.04	<0.01	<0.01	<0.01	<0.01	0.009-0.43	0.12	0.03
PCP	>0.14	-	0.36	-	<0.0001	<0.0001	<0.005	<0.005	<0.005	<0.005	0.0001-0.007	0.0033	0.01

As is evident from Table 1, the sediment quality objective is less than the lowest AET for all characteristics except chromium and nickel, thus achieving the first principle outlined above. For chromium and nickel, the values measured at the Puget Sound reference sites exceed the lowest AET by factors of about five and two, respectively. For chromium, the criterion established for Puget Sound is ten times the AET. No nickel criterion was established for Puget Sound. The sediment quality objectives established for Burrard Inlet for both these metals is about twice the lowest AET. These objectives equate to approximately the maximum value measured in the Fraser River and Boundary Bay, and the mean value for the Burrard Inlet reference site.

For the other metals, the general principle followed was to initially set the objective to the mean (or for arsenic, the mid-point) of the range of values for the Burrard Inlet reference site. If this first iteration yielded an objective below the actual data for the other B.C. reference sites, no further iterations were performed. This is what resulted for arsenic, nickel, and zinc. For cadmium, this concentration was too low relative to the maximum value for Loughborough Inlet or Boundary Bay. In this case, a value was selected for which 49 of 50 values for Boundary Bay were below.

For copper, lead, and mercury, the mean concentration for the Burrard Inlet reference site is extremely high relative to the other B.C. sites or the Puget Sound reference site. The sediment quality objective for copper is 100 µg/g, determined on the basis of being about twice the level at the Puget Sound reference site, and greater than all B.C. values except those in Burrard Inlet. The objective for lead is exactly 10% of the lowest AET, is the maximum concentration for B.C. sites except those from Burrard Inlet, and is the value listed for the Puget Sound reference site. The sediment quality objective for mercury of 0.15 µg/g is one-half the sum of the Puget Sound reference site concentration and the mean Loughborough Inlet concentration.

Table 2 illustrates the ratios of the sediment quality objective to the mean concentrations at the reference sites, as well as to mean concentrations at four other coastal British Columbia reference sites (Barkley and Laredo sounds, Surf Inlet, and Hecate Strait). When the ratios have a large range, a large degree of uncertainty exists for the objective.

TABLE 2
MEAN CONCENTRATIONS AND RATIOS OF SEDIMENT OBJECTIVES TO CONCENTRATIONS FOR REFERENCE SITES

Char-acter-istic	LOUGHBOR-OUGH INLET (LI) x	FRASER RIVER (FR) x	BOUNDARY BAY (BB) x	BURRARD INLET (BI) x	OBJ-ECT-IVE	RATIOS (:1.0)			
						OBJ/ L.I.	OBJ/ F.R.	OBJ/ B.B.	OBJ/ B.I.
As	<8	<6.6	3.6	-	20	2.5	3.0	5.5	-
Cd	0.44	0.29	0.17	0.52	1.0	2.3	3.5	5.9	1.9
Cr	36	40	27	57	60	1.7	1.5	2.2	1.1
Cu	64	38	11	190	100	1.6	2.7	8.8	0.5
Pb	22	17	5.4	48	30	1.4	1.8	5.6	0.6
Hg	0.12	0.08	0.026	0.23	0.15	1.3	1.9	5.9	0.7
Ni	22	43	18	43	45	2.1	1.1	2.5	1.1
Zn	115	90	46	150	150	1.3	1.7	3.3	1.0

Char-acter-istic	BARKLEY SOUND(BS) x	LAREDO SOUND(LS) x	SURF INLET (SI) x	HECATE STRAIT(HS) x	OBJ-ECT-IVE	RATIOS (:1.0)			
						OBJ/ B.S.	OBJ/ L.S.	OBJ/ S.I.	OBJ/ H.S.
As	-	-	-	-	20	-	-	-	-
Cd	1.3	1.05	1.09	0.38	1.0	0.8	0.9	1.0	2.6
Cr	49	35	27	29	60	1.2	1.7	2.3	2.1
Cu	37	36	31	4.3	100	2.7	2.8	3.3	2.3
Pb	7	12	7.7	12	30	4.3	2.6	3.9	2.5
Hg	0.095	0.2	0.13	0.05	0.15	1.6	0.75	1.2	3.0
Ni	-	-	-	-	45	-	-	-	-
Zn	124	100	75	33	150	1.2	1.5	2.0	4.5

The largest degree of uncertainty is present for the objective for copper, with ratios from 0.5:1 to 8.8:1. This range of ratios indicates that there are values both considerably higher and lower than the objective of 100 µg/g. Only the mean copper value for the Burrard Inlet reference site is actually higher than the proposed sediment objective.

The smallest degree of uncertainty exists for the objectives for chromium and nickel, for which ambient concentrations were above the lowest AET, and where the ratios were used as a means to estimate objective levels. Ratios for the other metals generally were between 0.5:1 and 5.9:1, although the exclusion of the Burrard Inlet ratios ensured that the ratios for the true reference sites were always greater than 1.0:1.0.

The sediment quality objectives for PCBs of 0.03 µg/g and chlorophenols of 0.01 µg/g (Table 1) are in-place in the lower Fraser

River (Swain and Holms 1985). The objective for PCBs has been shown to be plausible using a different procedure based on AET, baseline concentrations, and bioaccumulation potential (Harding 1989). The objective for chlorophenols applies to the sum of tri-, tetra-, and penta-chlorophenol and is 0.07 times the lowest AET for pentachlorophenol alone.

A different procedure was used to develop the sediment quality objectives for PAHs since the data base for the PAHs was considerably smaller and there were no data for either the Puget Sound or British Columbia coastal reference sites. The sediment quality objectives are in Table 3. The general principle followed was to establish the objective at a value of 0.1 times the AET (safety factor of 10:1), as long as this value was at least two to three times the analytical detection limit for the PAH.

TABLE 3
SEDIMENT OBJECTIVES FOR BURRARD INLET AND PAH DATA FOR REFERENCE SITES
(µg/g dry-weight)

PAH	LOWEST AET	FRASER R.		BOUNDARY BAY		BURRARD INLET		OBJECTIVE
		Range	x	Range	x	Range	x	
Σ LPAH	5.2		0.05		0.127		0.31	0.5
naphthalene	2.1	<0.005-0.13	0.018	<0.005-0.25	0.015	<0.01-0.11	0.05	0.2
acenaphthylene	0.56	<0.005-0.06	0.015	<0.005-0.62	0.036	<0.01-0.02	0.01	0.06
acenaphthene	0.5	<0.005	<0.005	<0.005-0.08	0.009	0.01-0.02	0.015	0.05
fluorene	0.5	<0.005	<0.005	<0.005-0.08	0.021	0.03-0.07	0.045	0.05
phenanthrene	1.5	<0.005	<0.005	<0.005-0.55	0.027	0.12-0.15	0.13	0.15
anthracene	0.96	<0.005-0.05	0.01	<0.005-0.10	0.019	0.03-0.04	0.04	0.10

PAH	LOWEST AET	FRASER R.		BOUNDARY BAY		BURRARD INLET		OBJECTIVE
		Range	x	Range	x	Range	x	
Σ HPAH	12.0		0.279		0.606		1.29	1.2
fluoranthene	1.7	<0.01-0.085	0.018	<0.01-0.91	0.164	0.19-0.23	0.21	0.17
pyrene	2.6	<0.01	<0.01	<0.01-0.3	0.03	0.19-0.24	0.21	0.26
benzo(a)anthracene	1.3	<0.01	<0.01	<0.01-0.07	0.089	0.12-0.14	0.13	0.13
chrysene	1.4	<0.01	<0.01	<0.01-0.15	0.018	0.21-0.27	0.24	0.14
benzofluoranthenes	3.2	<0.04-0.17	0.073	<0.04-0.47	0.077	0.21-0.29	0.24	0.32
benzo(a)pyrene	1.6	<0.02-0.098	0.028	<0.02	<0.02	0.06-0.15	0.095	0.16
indeno(1,2,3-cd)pyrene	0.6	<0.02-0.32	0.05	<0.02-0.40	0.038	0.02-0.09	0.06	0.06
dibenzo(a,h)anthracene	0.2	<0.02-0.37	0.06	<0.02-1.24	0.119	0.01-0.04	0.03	0.06
benzo(g,h,i)perylene	0.7	<0.02	<0.02	<0.02-0.65	0.051	0.08-0.13	0.10	0.07

For only one PAH (dibenzo (a,h) anthracene) did the 10:1 safety factor result in a value at the detection limit. For this PAH, the objective was set at three times the analytical detection limit, or at 0.06 µg/g (dry-weight).

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SEDIMENT QUALITY CRITERIA WORKSHOP SUMMARY

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1.0 Introduction

The workshop on Sediment Quality Guidelines, held at the 17th Annual Toxicity Workshop was organized by Dr. Margaret Taylor, D.o.E., in response to the need to develop adequate sediment quality guidelines. The following summary provides an overview of the workshop. The first section reviews the various approaches that were presented for establishing sediment quality guidelines. The second section provides a summary of the concepts behind each of the approaches. This is followed by a brief discussion of the advantages and disadvantages of each approach. The review concludes by suggesting some additional factors which weren't raised at the workshop that should possibly be taken into consideration when sediment quality guidelines are being established.

2.0 Approaches presented

In all, 6 different approaches for the development of sediment quality guidelines were presented.

1/ Equilibrium partitioning (Ep); C. Zarba, U.S. E.P.A.
2/ Apparent Effects Threshold (AET); R. Albright, U.A. E.P.A.
3/ Sediment Quality Triad (SQT); R. Dexter, E.V.S.
4/ Preponderance of evidence approach; E. R. Long, N.O.A.A.
5/ Spiked Sediment Bioassays; J. Ward, Battelle Marine Sciences Laboratory
6/ Burrard Inlet Approach (application of AET), L.G. Swain, B.C. M.o.E.

3.0 Summary of main concepts behind each approach.

3.1 Equilibrium partitioning (Ep): The Ep approach incorporates the EPA water quality criteria together with a correction factor for effects of organic carbon, i.e. sediment quality criteria (SQC) (ug/L) = K * water quality criteria (ug/L) * organic carbon content of the sediment; where K is the partition coefficient in L/kg for the chemical under analysis and organic carbon content is in percent. This approach has been developed specifically for non-ionic contaminants. Currently, the U.S. E.P.A. is attempting to develop methods for establishing sediment quality criteria for metals, using the acid volatile sulphide (AVS) content of the sediments as an indicator of sediment metal toxicity. At present, no method is available for establishing SQC for ionic organics, however, these compounds apparently comprise only a small part of the contaminant problem.

3.2 Apparent Effects Threshold (AET): The AET relies on a substantial data based on observed relationships between levels of sediment contamination and associated biological effects. It is defined as the concentration of sediment bound contaminant at which statistically significant biological effects are always observed (relative to appropriate reference sediments).

3.3 **Sediment quality Triad (SQT):** The SQT involves the collection of three synoptic measures of sediment contamination to determine a/ level of sediment contamination, as measured by sediment chemistry, b/ toxicity, as measured by sediment bioassays and c/ alteration of existing benthic communities, as measured by "in-situ" biological effects. Pooling of the three levels of information then allows for the identification of problem or degraded areas.

3.4 **Preponderance of evidence approach:** Relies on pooling data from a number of exiting SQC methods (e.g Ep and AET), over many geographical areas and determining the concentrations of contaminant where toxic effects either begin (effects range low) or where effects would be expected (effects range medium).

3.5 **Spiked sediment bioassays:** Based on the toxicity of a sediment as measured by percent survival of an organism, given a certain level of contaminant. In the method presented in the workshop the influence of sediment stability on sediment toxicity was addressed. Results of the sediment bioassay revealed that sediment toxicity varied with sediment stability; increased sediment stability inversely correlated with sediment toxicity. It was noted that the influence of sediment stability was not currently being incorporated into exiting methods of setting sediment quality guidelines, i.e. sediment toxicity was being established for stable sediments only.

3.6 **Burrard Inlet Approach:** This approach was an application of the AET approach for setting sediment quality objectives. Essentially, the general principle followed in developing these was, if possible to set the objective below the lowest measured AET from Puget Sound, while using data for B.C. reference sites to reflect local conditions.

4.0 Summary of advantages and disadvantages for each approach

4.1 Ep: The most attractive feature of the Ep approach is that it is based on chemical theory. Further, it uses already existing data bases. As well, uncertainties can be explored and identified allowing for confidence limits or safety factors to be included in the final SQC. The major drawback of the Ep approach is that it is chemical specific and therefore does not allow for possible synergistic effects among sediment bound contaminants. Further, as it relies on existing water quality criteria (i.e. SQC = K * WQC * % OC), if no WQC value exists for a particle contaminants then no SQC can be set. (e.g. PAH's).

4.2 AET: The main advantage of this approach is that the mechanism of interaction between the organism and the contaminant need not be known. Further, once the data base for determining AET has been developed, it can generate criteria values for all contaminants. The major draw back of this approach however, is the requirement of a large data base to establish the guidelines. Further, this approach cannot separate out single contaminant effects from the effects of multiple contaminants combined.

4.3 SQT: Like the AET approach, the mechanism of interaction between toxicant and organism need not be known. Further, this approach incorporates both single and multiple sediment bound contaminants. Finally, it provides empirical evidence for sediment quality. However, like the AET, it also requires a large data base. As well, no "statistical" criteria for the approach has been developed.

4.4 Preponderance of evidence approach: One of the main advantages of this method is that it allows for determination of confidence guidelines for the chemicals identified. However, as it relies on a number of existing data bases, it is labour intensive and is not clear for all chemicals (i.e. DDT and Cr).

4.5 Burrard Inlet approach: As this was an application of the AET approach, the same advantages and disadvantages which were outline for the AET would apply in this case as well.

4.6 Spiked bioassays: The main advantage of spiked sediment bioassays are their use in determining dose-response relationships. The main disadvantage of course is attempting to apply these toxicity values to the "real" world.

5.0 Summary and conclusions

There is an urgent need to develop sediment quality criteria for the protection of public health and the environment. As to which method holds the most promise for the development of these guidelines has not been established. No one method has yet to be embraced as being the "best" method. There is a genuine effort however, among the agencies behind each of the approaches to work together to try and determine the "best" method of setting sediment quality guidelines.

To date the focus of the approaches has been on bottom sediments. There has been little or no attempt to address the importance of contaminated suspended particulate matter. In the case of some filter feeders, this source of material can be the main vector of contamination, rather than that which is obtained from the sediments directly. Hence quality guidelines for particulate matter as with sediment quality guidelines are also urgently needed. Finally, once a method for setting sediment quality guidelines has been established, this method has to be accessible and affordable to all, especially developing countries who cannot afford to allocate monies to addressing problems of sediment and water quality.

WORKSHOP

Sediment Toxicity Bioassays

Chair: T. Thompson

SEDIMENT TOXICITY TESTS: DO THEY TELL THE "TRUTH"?

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Sediment toxicity tests are routinely used to assess the hazards associated with contaminated sediments. In the regulatory context, these tests are meant to be predictive of the level of effect to be found in the benthic community under natural conditions. There are several examples of instances where evidence of toxicity in the laboratory is not accompanied by concurrent observations of deleterious effects in the benthic community. Conversely, there may be cases of observed effects in the benthos that are not confirmed by sediment toxicity.

There are several reasons for the lack of concurrence between toxicity and abundance/community data. Complete concordance between toxicity test results and the condition of the benthos may not be achieved for several reasons, which include: contaminant bioavailability and the role of organic carbon and sulfide pools; toxicity due to grain size, ammonia or other non-contaminant factors; and the behavior of infaunal organisms, and how they modify exposure.

A simple explanation is behavioral, where the infauna may avoid the contaminated portions of the sediment column, as was demonstrated for the polychaete Nephtys incisa when exposed to layered contaminated sediments (Johns et al. 1985). This would be the case when a contaminated sediment is gradually layered over by natural deposition allowing for normal recruitment of the infauna. Often, the sediment for toxicity testing is collected to depth and the toxicity test organisms are exposed to an homogeneous mixture that would cause toxicity. It is also possible that only larger members of the population would be exposed to the deeper sediment layers and this behavioral effect would only be observed in older populations.

Organic carbon content is known to be a factor controlling the availability of organic and inorganic contaminants. Initial evidence indicates that acid volatile sulfides may play a similar role in controlling the availability of metals (Ditoro et al. in press). Normalization procedures have been used to express contaminant concentrations in relation to modifying factors, but the relationship to toxicity remains to be quantified.

When sediments are collected and homogenized, the vertical distribution of organic carbon and sulfides, as well as their character, may change significantly. For example, sulfides may be oxidized releasing bound metals into the pore waters for exposure during the test. Thus, laboratory and field exposures would be very different.

Another potential cause of discrepancies between toxicity and benthic data is that some toxicity may be expected in the absence of contaminants. This toxicity may result from sensitivity to variations in grain size, or from exposure to ammonia and hydrogen sulfides released from some types of sediments under static exposures. These types of effects on amphipods used in toxicity tests has not been quantified and cannot be ruled out as a cause of toxicity.

Lastly, the relative sensitivity of the more commonly used species or test systems is not well understood. Amphipod sensitivity appears to be in the mid-range of the species that have been tested in the EPA water quality criteria program. It is unknown how this sensitivity compares with that of typical benthic organisms. Also, testing programs using multiple species are favored over reliance on the response of a single species.

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SLAG, BENTHOS, AND BIOASSAYS: POOR CORRELATION OF BIOASSAY PREDICTIONS AND THE BENTHOS.

R. L. Shimek, T. Thompson, and D. Weitkamp, Parametrix, Inc. 13020 Northup Way, Bellevue, WA., U. S. A. 98005, (206-455-2550).

We examined relationships between two marine sediment bioassays and benthic parameters using sediments containing slag. Metals (As, Cu, Hg, Pb, Zn) concentrations ranged from 52 to 44,300 ppm. Wet-weight biomass ranged from 13.5 g/m^2 to 257.3 g/m^2, the number of species, ranged from 45 to 151, and the mean number of individuals/m^2, identified to species, ranged from 248 to 10,860.

Amphipod (*Rhepoxynius abronius*) 10 day survival, and echinoderm (*Dendraster excentricus*) embryo survival bioassays were compared with biomass and abundance for four taxonomic groupings: Annelida, Arthropoda, Mollusca, and Miscellaneous (all other) Taxa. Additionally abundances of particular taxa within these major taxa were compared. We also compared bioassay results with the total number of taxa at each station, and ecological indices (H', C, and J).

We found few significant correlations between the station bioassay results and station biological parameters. We conclude that with such sediments, these bioassays are inappropriate and give misleading responses.

SEDIMENT BIOLOGICAL TESTS: REGULATORY INTERPRETATION IN THE "GREY AREA." K. Phillips, Washington Department of Ecology, Mailstop PV-11, Olympia, WA, U.S.A. (206) 459-6143.

Washington Department of Ecology (Ecology) has proposed a new state rule that establishes sediment quality standards consisting of chemical concentration criteria, biological tests and test interpretation for marine sediments. The rule also details the applicability of these standards to discharge source control and sediment cleanup programs. A separate rule is being prepared that establishes requirements for dredging and disposal of contaminated sediments in water, shoreline and upland environments. Both of these rules combine sediment chemical measurements and direct biological testing of sediments to establish regulatory requirements for managing sediments.

Testing requirements are established in a tiered process, beginning with initial sediment chemical testing and application of sediment chemical criteria. The second tier consists of biological testing where needed, to include acute and chronic effects testing. Standard biological tests are applied for acute effects testing; biological testing for chronic effects is addressed through surrogate acute tests, field evaluation of the benthic community and/or a recently developed laboratory growth test. Test interpretation combines chemical and biological measurements to reduce overall decisionmaking uncertainty and to ensure an efficient regulatory process. Ongoing efforts to develop freshwater and human health sediment criteria are in progress.

UNDERSTANDING SEDIMENT TOXICITY OFF SOUTHERN CALIFORNIA

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SUMMARY

Benthic species composition and abundances change along sewage outfall gradients off southern California (Word and Mearns, 1979; Swartz et al., 1986). The most contaminated areas are inhabited by low diversity assemblages dominated by the polychaete Capitella capitata. Normal, or reference areas are dominated by the ophiuroid Amphiodia urtica and the urchin Lytechinus pictus, neither of which exist in the contaminated areas. Why these species do not inhabit contaminated sediments is not known.

In trying to understand cause-and-effect of these patterns, we have utilized empirical field studies and experimental laboratory studies focusing on key, indigenous benthic species. Laboratory toxicity tests have shown that whole sediment from contaminated areas caused mortality and reduced growth to both Lytechinus and Amphiodia, but we do not know which component(s) of the sediments caused the mortalities (Thompson et al., 1989; SCCWRP unpubl.). Similarly, field studies of benthic macrofaunal assemblages were inconclusive (Anderson et al., 1988). The problem with both of these approaches was that the numerous contaminants present in sediments generally covaried; where one contaminant (including organic material) was high all were high. Even multivariate ordination methods could not correlate individual contaminants with the observed biological effects. We concluded that it is not possible, using whole sediment mixtures, to determine which contaminants, let alone what concentrations, cause biological responses.

Our current approach is to conduct laboratory flow through exposures using clean, natural sediment from reference areas, spiked with varying concentrations of a single contaminant. In the phenanthrene exposure we used the amphipod Grandidierella and measured growth, mortality and bioaccumulation for 14 d. Rapid microbial degradation of phenanthrene in the sediment (55% in 2 d), precluded precise toxicity limits and no significant growth or mortality effects were observed at phenanthrene levels up to 30 ppm (dry wt.) (Bay, 1989).

Since dissolved sulfides were identified as a potential effector in our previous experiments using whole sediments, we exposed Lytechinus in aquaria designed to have elevated sulfide in the pore water, but without the confounding

effects of elevated organic material. We showed consistent effects on growth, mortality, and gonad production at H_2S pore water concentrations above 8.6 mg l^{-1} (Thompson, Bay et al. ms.) These H_2S concentrations are similar to the levels that caused significant responses in our previous experiments.

There are also problems with this approach. Lab exposures are labor intensive and the list of possible contaminant effectors is very long. Thus, working through all the comparative toxicity testing would require a very long time. Additionally, critical sediment levels identified in laboratory exposures require field validation. We are particularly interested in what our laboratory results may mean in the actual receiving waters. We have focused on making ecologically meaningful measurements in our toxicity measurements, in particular, growth, mortality and gonad production. These measures are all components of most population growth models and therefore may be used together to create predictive models of population consequences of contaminant exposure (SCCWRP, unpubl). Together with information from complementary field studies, we are attempting to produce such models.

Another approach that may be useful in determining which sediment contaminants cause biological responses is being pursued in our study of the recovery of Santa Monica Bay from 30 years of sludge discharge (Thompson and Dorsey, 1989). As recovery proceeds, concentrations of the various contaminants in the sediment are decreasing at different rates. At the same time we are observing benthic macrofaunal responses (species composition, abundances) at different rates. Using multivariate pattern recognition methods, we believe that it will be possible to separate out which contaminants are keying the recolonization of the benthos. This field validation will provide a valuable test for what we learn in our laboratory exposures.

In summary, we do not believe that is possible to determine from laboratory or field studies alone which contaminants, or their concentrations, cause benthic toxic effects. It is only through iteration of both lab and field studies that valid sediment toxicity thresholds can be determined. Regulatory agencies must allow the time, and provide the resources to properly determine and validate sediment quality objectives.

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SUMMARY OF THE SEDIMENT TOXICITY BIOASSAY WORKSHOP

17th ANNUAL AQUATIC TOXICITY WORKSHOP

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The goal of the workshop was to examine the utility of sediment bioassays within the framework of the triad approach to contaminated site assessment. While sediment bioassays, in conjunction with sediment chemical analysis, are important predictors of impacted sites, to infrequently are *in situ* measurements made of the benthic communities to determine if the results of the bioassays and chemical analysis are environmentally relevant. Where those measurements have been made, the results may be in concordance, or contradictory. The workshop chose to discuss examples of those situations, scientific interpretation of concordant and contradictory data, and regulatory use of triad-generated data.

Dr. John Scott, of Science Application International Corporation in Naragansett, RI, presented introductory comments and a general review on observed correlations between the three elements of the triad. Working with the theme of relevance of sediment bioassays as indicators of sediment community health, examples were given on both the adequacy and inadequacy of sediment bioassays impact predictors. These included studies were presented where bioassays predict impact where none existed, or where the other two elements of the triad demonstrated impacts, but the bioassays failed to predict those. Dr. Scott offered the following four areas for discussion as to what mitigating circumstances can effect the three elements of the triad:

Contaminant bioavailibility. Conventional chemical analysis could show high levels of chemical contaminants, but the toxicity tests and benthic communities show no apparent effect. Conversely, chemical analysis may show low levels of contaminants, but the benthic community and bioassays show severe impact. Factors contributing to bioavailibility in sediments, such as total organic carbon, resident sulfide pools, and sample collection techniques, were discussed.

Benthic infauna avoidance of contaminants. Examples were presented in which benthic communities avoided the contaminated sediments. The communities could be found existing above or below the contamination layer. Thus while bioassays and chemical analysis would predict impact, the benthic data would indicate a healthy, thriving community.

Relative sensitivities of the bioassay tests . Toxicity testing is still relatively new, and there is yet insufficient knowledge concerning the tests and organisms used. In the currently available tests, some species may be too sensitive, or tolerant of contaminated sediments. A general lack of good chronic indicators hampers our ability to make adequate predictions concerning long-term impacts.

Physical or non-contaminant effects . The test organisms used in the bioassays may be sensitive to physical effects, or non-contaminant toxic within the test sediments. Examples include sediment grain size effects, or the presence of high ammonia levels that could cause toxicity in some test organisms.

Dr. Ron Shimek, of Parametrix in Bellevue, WA, presented a case study using the triad approach to an off-shore slag pile at the ASARCO copper refinery in Commencement Bay, WA. As part of an on-going EPA Superfund directed Remedial Investigation and Feasibility Study, the results indicated conflicting trends between the chemical and biological parameters. These included:

High levels of heavy metals in the slag. Chemical analysis showed that for combined metals (arsenic, copper, mercury, lead, and zinc) concentrations ranged from 42 to 44,300 ppm.

Bioassay toxicity shown throughout the site. Using the larval echinoderm and oyster tests, as well as the 10-day acute *Rhepoxinius abronius* sediment exposure system, toxicity was demonstrated for a number of sites. Correlative analysis showed a positive relationship between total metals, and *Rhepoxinius* mortality, but there were no statistical correlations were found between the larval tests and total metals.

Presence within the slag of an abundant and diverse benthic assemblage. Using indicators such as total animal numbers, species abundance and diversity indices, and total biomass, the benthic community found within the slag pile was greater than those found in nearby, uncontaminated reference areas. Correlative analysis could not demonstrate a relationship between metal concentration and infaunal community health.

No relationship between toxicity bioassays and benthic infaunal communities. Using correlative analysis, there were no statistically significant relationship between bioassays and the infauna. In this study, bioassays were not adequate predictors of impacts.

Keith Phillips of the Washington Department of Ecology (Ecology) discussed application of biological and chemical criteria in establishing regulatory requirements for sediments. In Washington, two separate programs have been promulgated establishing criteria for open water disposal of dredge material spoils, and for defining and managing marine sediment quality. Use of the components of the triad in the program are as follows:

Puget Sound Dredge Disposal Analysis. The PSDDA program is an interagency cooperative program that has set the criteria for defining when a dredge spoil is suitable for open water disposal at a state-operated disposal site. In cooperation between Ecology, EPA, the U.S. Army Corp of Engineers, and the Washington Department of Natural Resources, the program used the Accumulated Effects Threshold method for calculating limits for chemicals of concern (COC). A tiered process of analysis was implemented that first requires chemical analysis of composites collected within the dredge footprint. Using AET's, screening levels and maximum levels for the chemicals of concern were established. If analysis does not exceed any of the COC screening levels, the sediments are suitable for open water disposal. If the analysis shows COCs above screening levels, but below maximum levels, biological testing using the acute *Rhepoxinius abronius*, mollusc or echinoderm larval survival, acute juvenile polychaete, and Microtox tests for establishing suitability for disposal of the sediment. Benthic infaunal analysis were not included in this program due to the confounding issue of harbor channel scouring impacts to infaunal communities.

Washington State Marine Sediment Quality Standards. Ecology has formulated a state rule that sets biological and chemical criteria for marine sediment quality. The rule is will be used for within the state's marine effluent discharge program to define allowable levels of sediment impact caused by industrial and municipal dischargers. Furthermore, it establishes measurement and cleanup standards for existing impacted marine sediments. As with the PSDDA program, the standards utilize AET-derived chemical trigger values, and defines toxicity testing limits. Unlike the PSDDA program, the rule is effects-based in that toxicity tests may occur prior to chemical analysis. In the rule, all three elements of the triad are incorporated, but any one of the three elements may be used as a sole classifier for the test sediment; i.e., a significant response in either bioassays, chemistry, or benthic analysis may define a sediment as contaminated. The rule does maintain a sufficient degree of flexibility that would allow for consideration of all three elements of the triad, on a case-by-case basis.

Dr. Bruce Thompson, of the Southern California Coastal Water Research Program, presented results of on-going research to correlate the three elements of the triad along outfalls in southern California. The aim of that work is to use both spiked lab toxicity measurements, coupled with correlative infaunal analysis, to set valid sediment chemical criteria. To that end, the work at SCCWRP includes the following elements:

Empirical Field Benthic Infaunal and Chemical Analyses. Within the outfall fields gradient measurements of both infauna and chemistry have been taken along the sludge filed gradient and within reference areas. Statistical analysis designed to correlate individual contaminants with the observed biological effects have to date been unsuccessful.

Laboratory Toxicity Tests. Lab toxicity tests have demonstrated both acute and chronic toxicity of the whole outfall sediments to reference area species. In attempt to define which contaminants are causing toxicity, the lab has begun flow-through spiked sediments.

Monitoring of Recovery with the Santa Monica Sludge Field. As the outfall at Santa Monica is now non-operational, the area is expected to begin recovery. Monitoring is continuing, and the sludge fields are being watched to determine if declines in certain chemicals can be correlated with appearance of reference area benthic infauna. Correlative analysis between these field observations and spiked laboratory tests will yield information toward developing sediment quality standards.

In the post-presentation discussion, participants discussed within a triad analysis, and the relative weight that should be accorded to each of the elements. It was agreed that a triad simply represents a method for collecting information using chemistry, bioassays, and benthic infaunal analysis. The relative weight accorded to each element will depend upon the specific program, but it was generally acknowledged that benthic infaunal analysis is the most reliable indicator of sediment quality. Continued refinement and analysis needs to be conducted to achieve increasing confidence in chemistry and bioassays as predictive indicators, and regulatory tools. The work group expressed the need for continuing refinement of sediment quality standards, and that the approach taken by Dr. Thompson would be a useful approach for both the Washington state regulatory program instrumental in further refinement of sediment quality standards. The risk assessment approach that is usually used in upland contamination studies was also listed as an approach that should deserve further research.

WORKSHOP

Sediment Quality Criteria Values

Chair: P. Ross

Panelists:
R. Barrich
L. Hare
D. Hart
C. Ingersoll
E. Long
W. Schneider
C. Zarba

17TH AQUATIC TOXICITY WORKSHOP

Vancouver, Canada
5-7 November 1990

Synopsis of a colloquium on:

SEDIMENT QUALITY CRITERIA: A LOOK AT THE DATA

Philippe Ross, Session Chair

Rationale:

There is inexorable pressure from various levels of government to develop Sediment Quality Criteria. The implications of this are enormous, as these criteria will eventually drive regulations, enforcement actions, dredged material disposal options, and the setting of post-cleanup target conditions for remedial actions. At least a dozen methods have been proposed for calculating these criteria, and the big question is 'Which method or combination of methods should be used?' Several efforts at calculating criteria have been undertaken, so it was appropriate to begin to compare criterion values obtained for different chemical species, by different investigators, using different methods, and employing different data bases. The objective was to obtain a relative idea of what to expect from each of the proposed methods, and to stimulate discussion.

Format:

The workshop organizers opted for a panel-discussion format with panelists chosen to represent different viewpoints in Canada and the United States. Prior to the workshop, panelists had submitted lists of calculated values for entry into a computer file. The session began with a five-minute statement from each panelist describing the calculation method and data base used. The session was then opened to questions from panelists and the audience. This discussion was aided by an interactive graphical display of values and comparisons (MacIntosh computer, CricketGraph software, Sharp QA-50 projection system) In this way, everyone attending had a clear picture of the differences and similarities among the numbers presented, and suggested plots and comparisons could be quickly displayed.

Panelists:

The colloquium was convened and chaired by Philippe Ross, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign, IL 61820, USA. Eight panelists were invited, four from Canada and four from the United States. The names and affiliations of the panelists are listed below, along with the method(s) the use to generate sediment quality values and the data base(s) they work on.

Panelists:	Methods	Data bases
Barrick, Robert C. PTI Environmental Services 15375 SE 30th Place, Suite 250 Bellevue, WA 98007, USA	Apparent Effects Threshold	US West Coast
Hare, R. Landis Université du Québec, INRS-EAU 7500, rue Einstein Ste-Foy, QC, CANADA G1P 3W8	Long-term in situ spiked sediment communities	Lac Tantaré (Québec)
Hart, Don. R. Beak Consultants Limited 14 Abacus Road Brampton, ON, CANADA L6T 5B7	Background; Equilibrium Partitioning; Apparent Effects Threshold; Screening Level Concentr.; Spiked Bioassay	Great Lakes
Ingersoll, Christopher G. National Fisheries Contaminant Research Center U.S. Fish and Wildlife Service 4200 New Haven Rd., Columbia, MO 65201, USA	Apparent Effects Threshold	Great Lakes
Long, Edward R. National Oceanic and Atmospheric Administration Ocean Assessments Division 7600 Sand Point Way NE, Seattle, WA 98115, USA	Effects Ranges Based on Consensus of Available Data (ERBOCOAD)	Global
Scheider, Wolfgang Ontario Ministry of the Environment Water Resources Branch, 1 St. Clair Ave. W., Toronto, ON, CANADA M4V 1K6	Equilibrium Partitioning Screening Level Concentr.	Great Lakes Basin
Smith, Sherri Water Quality Branch, Environment Canada P.V.M., 351 St. Joseph Blvd., 7th floor Hull, QC, CANADA K1A 0H3	Effects-based; Background; Equilibrium Partitioning	Global
Zarba, Christopher USEPA, Criteria Standards Division, WH-585 401 M Street, S.W. Washington, DC 20460 USA	Equilibrium Partitioning	National

Results:

The session was convened at 1:30 PM on Tuesday, 06 November 1990 in the Garibaldi Room of the Hotel Vancouver. Discussion moved between panelists, the audience and the convener, and covered a broad range of issues, including philosophical issues underlying the setting of sediment quality guidelines. After four hours of discussion and data comparison, the following points were unanimously agreed upon by the panel:

1) *In any guideline-setting exercise, it is absolutely essential that values be normalized for factors that influence bioavailability.* Total Organic Carbon content of the sediment (TOC) and Acid Volatile Sulfide content (AVS) are two important normalizing factors. Sediment quality concentrations that are not normalized are of little or no value. Data bases without sufficient data for normalization should not be used to set criteria.

2) *It is highly desirable to apply a variety of approaches to each criterion-setting situation.* As different methods tend to produce different sediment quality values, results of only one method can be misleading. Using more than one method increases the likelihood that management decisions will be protective of the environment.

3) *When comparing the relative performance of two guideline-setting methods, the chemicals used in making the comparison should be representative of all areas used in the data base.* There is a possible bias due to radically different sets of concentrations or unequal rates of occurrence in different data sets.

4) *We must be cautious with criteria development.* Once guidelines are in place, they will inevitably be used in enforcement or regulatory actions. The first legal challenges to these guidelines will be crucial in determining the ultimate success or failure of sediment quality regulations. we should proceed only in cases where we have a great deal of confidence in the numbers. It is better to be realistic about what we do know and what we do not know.

5) *In setting and applying sediment quality guidelines, the combined use of chemical and biological information is indispensable.* The two types of information should be balanced when making decisions and recommendations.

6) *Guideline-setting approaches should be flexible.* Wherever the option exists, contamination as indicated by chemical analysis should be verified by biological information before regulatory decisions are made.

7) *The approach used in the NOAA Status and Trends report (Long and Morgan 1990) is a useful one.* Looking at the distribution of a wide array of effects data helps to situate calculated sediment quality values along the response continuum, and could help support arguments based on preponderance of evidence. The NOAA report is a valuable resource and should be periodically updated.

8) *On a national or global scale, independent verification of guideline values is necessary.* Sediment quality concentrations produced from one data set should be independently verified with another data set in order to develop confidence in the reliability of the values. Retrospective analysis of previous data in light of new information should be encouraged.

9) *It is not enough to issue lists of numbers.* There is always a danger that quality guidelines will be taken out of their original context and improperly applied. This potential can be minimized if guidelines are always accompanied by clear explanations of what can and what cannot be done with the numbers.

10) *When reviewing data sets to eliminate unrepresentative values, one must have a clear understanding of the mechanisms involved.* Some editing and cleaning up of sediment quality data sets is inevitable and desirable. Data points that are clearly outliers should be removed before final guideline-setting calculations are done. No data should be deleted, however, unless a chemically or biologically defensible reason is given.

11) *The regulatory community is desperate for sediment quality criteria.* We will have to give them something, but we must take care that the job is done properly.

12) *Sediment quality guideline development has helped spark the growing interest in sediment toxicology.* It was observed that the recent increase in the numbers of papers and sessions devoted to sediments at SETAC and ATW meetings parallels the guideline development effort.

13) *Generally speaking, scientists working on guideline development are cooperating and sharing knowledge.* The rumors of division and factional infighting among key proponents of various methods were perceived by the panel as being greatly exaggerated.

The session was adjourned at 5:00 PM. The convener read a summary report of the colloquium at the concluding plenary session on Wednesday, 07 November 1990.

WORKSHOP

Indicators of Toxicity Stress

Chair: S. Adams

BIOLOGICAL INDICATORS OF TOXICITY STRESS. S.M. Adams,
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Various approaches have been used to evaluate or predict the effects of environmental stress on fish. The most common of these approaches are laboratory tests of acute and chronic toxicity and the measurement of either a single stress response or responses at only one level of biological organization. Although such approaches are valuable for addressing specific objectives, such as establishment of water quality criteria, they lack ecological realism. In addition, environmental stressors are more complex and subtle than the acute stressors applied in most laboratory stress studies. Consequently, application of such tests to the natural environment can often lead to incorrect evaluations and inaccurate predictions of chronic stress effects on fish. Approaches are needed, therefore, that (1) permit the detection of stress-related biologically and ecologically relevant variables, and (2) maximize predictive capabilities.

The effects of stress are usually manifested at lower levels of biological organization before disturbances are realized at the population, community, or ecosystem levels. Sublethal stress is generally expressed first at the molecular and biochemical levels through interference with enzymes, cell membranes, or genetic material. Such changes induce a series of structural and functional responses at the next higher level of biological organization. These induced responses can impair, for example, integrated processes such as hormonal regulation, metabolism, osmoregulation, and immunological regulation. These effects, in turn, may eventually affect the organism's ability to survive, grow, or reproduce. Ultimately, irreversible and detrimental effects may be observed even at the population, community, or ecosystem levels. In the sequence of biological organization from subcellular and cellular levels through populations to communities, each level of organization finds its functional explanation in the levels below and its significance in the levels above. By measurement of stress responses at various levels of biological organization, it should be possible to monitor a spectrum of sensitivities to stress, specific effects, and several points of ecological relevance simultaneously. Understanding of the relationship of these stress responses to each other using biological indicators should improve our capabilities for prediction of population and community changes before irreversible damage occurs.

Use of biological indicators (bioindicators) of toxicity stress differs from more traditional methods of monitoring the effects of pollutants on the environment (e.g., direct measurement of chemical pollutant levels or use of laboratory toxicity tests) by using naturally occurring organisms as sentinels or early-warning indicators of environmental damage. Since the physiological condition of an animal tends to integrate the effects of all the environmental stresses (such as contaminants, unfavorable temperatures, water velocity, suspended sediment, oxygen, and food availability) acting on it, measuring an appropriate set of bioindicators permits an assessment of the nature and extent of environmental degradation. Bioindicators can be used as early warning signals of stress, to establish cause-and-effect relationships between the different levels of stress response, and to evaluate the effectiveness of remedial actions on the biotic integrity of aquatic systems. Even though use of bioindicators in field situations have experienced some success, there is much to be learned relative to the evaluation and interpretation of their use such as: 1) major advantages and limitations of various groups of indicators, 2) application of different indicators under various types of toxicant stress, and 3) the influence of other environmental variables on the expression or manifestation of organism stress response. This workshop offers an ideal format to discuss and help resolve issues related to effective use and application of biological indicators of toxic response.

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WORKSHOP

Geochemical Aspects of Bioavailability I & II

Chair: A. Lewis

GEOCHEMICAL ASPECTS OF METAL BIOAVAILABILITY: AN OVERVIEW OF SEDIMENT GEOCHEMISTRY.

Bjorn Sundby, Maurice-Lamontagne Institute, Department of Fisheries and Oceans, Box 1000, Mont-Joli, Québec G5H 3Z4 (418-775-6703)

The main problem in determining the effects of contaminated sediments on benthic organisms is the difficulty of knowing precisely what the organisms are exposed to. The difficulty is not only related to analytical chemistry and speciation, but also to the heterogeneous and dynamic nature of the sedimentary environment. The concentration of a trace element in the pore water or in the bulk sediment, for example, can differ by orders of magnitude between the surface and a few centimetres depth. The vertical distribution of a trace element can also vary in time and space as a result of biological events in the water column and patchiness in the sediment. The benthic organisms themselves interact with the sediment and modify the chemical composition of their immediate environment. Construction and irrigation of burrows is one example. Mixing the sediment is another. Hence meaningful statements of exposure can only be made on the basis a clear understanding of the sedimentary environment and the organisms' relationship to it. In this paper I will give an overview of our present understanding of biogeochemical processes in fine grained sediments, based on studies of early diagenesis in the St. Lawrence estuary.

BIOAVAILABLE SEDIMENT-BOUND METALS: ESTIMATION BY CHEMICAL EXTRACTION.

E.A. Jenne, PO Box 999, Pacific Northwest Laboratory, Richland, WA, USA (509-375-6869)

Reliable estimation of the bioavailable quantity of a metal in sediments is important because metals present in the structure of minerals (e.g., silicates) or occluded by precipitates are essentially unavailable to organisms. Hence, total metal concentrations in sediments may bear little relationship to the metal content of associated aquatic organisms. "Selective" extractions are used to obtain information on the partitioning of metals among adsorbents or to assess the quantity of bioavailable metal present irrespective of the solid phase(s) with which the metal is associated. Preferential extraction of metals from individual major adsorbents is often attempted by adjustment of pH, E^H, and/or complexing agent concentration. The selective extraction of metals from sediment recovers metals simultaneously from multiple solid phases. This is a consequence of the fact that there is no point in three-dimensional pH, E^H, and complexing agent space that only one major adsorbent exhibits significant solubility. The success of acidic extractants in estimating the quantity of bioavailable metals may be due to the ability of H^+ to desorb metals from surface complexation sites, to partially dissolved carbonates, oxidic Mn and amorphic Fe, and to minimize re-adsorption during extraction. Pacific Northwest Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute under Contract DE-AC06-76RLO 1830.

ANALYTICAL METHODS FOR THE STUDY OF BIOAVAILABILITY

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ABSTRACT

An enzyme, bovine carbonic anhydrase, has been used to monitor the available zinc and copper in model systems. The enzyme has been used both in homogeneous systems and as the enzyme bound to controlled pore glass. The apo form of the enzyme was prepared by equilibrating the enzyme with 0.10 M dipicolinic acid at pH 7.5. Zinc activates the enzyme and copper inhibits the enzyme. The studies indicate that the sensitivity of the method depends on the equilibration time. Acceptable calibration curves are possible for pZn values of 10 to 8 and pCu values of 8.5 to 11. The enzyme fixed to the controlled pore glass was found to be reusable and more adaptable to turbid samples.

INTRODUCTION

Analytical methods for the determination of the bioavailability of metal ions in natural waters are based upon one of three assumptions:

1. the organism will equilibrate with the metal ions in the water sample. A simple model for the organism is a membrane (that is permeable to metal ions) that encloses a set of metal binding ligands. The metal ions in the water sample titrate the ligands. An effect on growth will be noted when a certain fraction of the ligand is titrated with either a nutrient (ie enhances growth) or a toxic substance (ie inhibits growth). If the **total** concentration of metal ions is high then the organism would ultimately equilibrate with the **free** metal ion concentration. This model has been demonstrated (Petersen, 1982) to be applicable to algae grown in synthetic media. Methods for the determination of the concentration of the free ion include potentiometry with an ion selective electrode and equilibration with ion exchange resins. The chemistry of the ion exchange method has been assessed by Sweileh et al. (1987) and Zorkin et al. (1986) have compared the ion exchange method with algal assays.

2. the membranes associated with the organism are permeable only to low molecular weight species. This assumption leads to a size fractionation procedure based either upon dialysis or ultrafiltration. These methods are often used to discriminate between the free ion and inorganic complexes as a potential bioavailable class and colloidal material (both organic colloids such as humic and fulvic acids and inorganic colloids) as a nonavailable class.

3. the organism is thought to interact with the surroundings to bind metal ions and create a "depletion layer" surrounding the organism. A metal ion complex is thought to be bioavailable if the species can dissociate to yield the free ion during the time that it is present in the depletion zone. The bioavailability is then controlled by the kinetics of the dissociation of the metal complexes. Analytically there are three kinetic regimes--a very labile species that can dissociate within milliseconds and measured using anodic stripping voltammetry (Florence, 1989), a pseudolabile species that dissociates on the time scale of seconds and measured using column flow techniques using either ion exchange resins or Chelex 100 (Figura and McDuffie, 1979) and nonlabile species. Muller and Kester (1990) have recently compared these procedures for zinc and cadmium in seawater. A number of workers (Olson and Shuman (1983), Lavigne et al. (1987), Cabaniss (1990), Hering and Morel (1990)) have used spectrophotometry and ligand

exchange techniques to determine the lability of metal ions in model colloidal systems.

The requirements of a good monitor of metal bioavailability are sensitivity to very low levels of metal ions, it should mimic the interaction of metal ions with the organism, aHx it should be suitable for multimetal measurements. An enzyme monitor of metal availability is attractive because it may satisfy these three requirements. This paper summarizes the application of the apoenzyme form of carbonic anhydrase as a monitor of copper and zinc speciation in model systems. One advantage to this enzyme is that the active site of the enzyme is stereochemically hidden from the surrounding environment--only certain species can interact with this site to either activate or inhibit the enzyme.

If the stereochemical requirements are stringent enough then the activation or inhibition of the enzyme can occur only through the free metal ion and the kinetics are controlled by the following scheme:

$$\begin{array}{c} ZnL \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Zn^{2+} + L^{2-} \\ + \\ ENZ \\ k_2 \downarrow\uparrow k_{-2} \\ ZnENZ \end{array}$$

An exact solution for the kinetics of this system is not possible but, if one assumes that the experiment is done under conditions where the free zinc ion is bufferred, then a steady state assumption results in the following relationship:

$$\frac{d[ZnENZ]}{dt} = k_2[ENZ]\left(\frac{k_1[ZnL]}{k_{-1}[L]+k_2[ENZ]}\right)$$

The final form of the kinetic equation depends on the relative magnitude of the two terms in the denominator. The value of k_2 for carbonic anhydrase and zinc ion is on the order of 10^4 $M^{-1}s^{-1}$ and a typical concentration for the apoenzyme in these experiments was 10^{-7} M giving a value of 0.001 for the second term. Typical values for [L] and k_{-1} are 10^{-5}M and 10^6 $M^{-1}s^{-1}$, respectively. This suggests, therefore, that the first term in the denominator is of the order of 10 and much larger than the second term. Under these conditions the rate of activation (or inhibition) of the apoenzyme would be controlled by the free metal ion concentration. This kinetic control of the activation process allows one to control the sensitivity of the monitor.

MATERIALS AND METHODS

Bovine carbonic anhydrase (EC 4.2.1.1), p-nitrophenolacetate, TRIS, and dipicolinic acid were purchased from Sigma Chem. Co. Aminopropyl coated controlled pore glass beads were purchased from Pierce Chem. Co. The glass beads were activated using glutaraldehyde and the carbonic anhydrase was fixed to the beads using standard procedures. All other reagents were reagent grade or better. All salt, buffer, and complexing agent solutions were passed through a 10 cm x 1 cm column of Chelex 100 column to remove trace metal impurities.

A stock solution of carbonic anhydrase was prepared by dissolving 0.100 grams of enzyme in 25 mL of distilled water. The apo form of the enzyme was prepared by mixing 1.0 mL of the stock enzyme with 1.0 mL of 0.10 M dipicolinic acid (adjusted to pH 7.5 using solid TRIS). After a 60 minute equilibration period 1 mL of the solution was passed through a 1.0 cm x 20 cm column of Fractogel

HW50F and eluted with a solution of 0.01 M citric acid/TRIS adjusted to pH 8 to separate the apoenzyme from the dipicolinic acid. The appropriate fractions were combined and diluted to 10 mL with TRIS buffer to give the working apo stock solution. The apo form of the enzyme fixed to the controlled pore glass beads was prepared by equilibrating the beads with 0.10 M dipic for 60 minutes, filtering the beads using 0.10 µm nylon membrane filters, and washing well with 0.010 M citric acid/TRIS pH 8 buffer.

The assay procedure for homogeneous systems consisted of adding 100 µL of apoenzyme to 25 mL of test solution, equilibrating for 10 to 60 minutes, and removal of 3.0 mL of solution for measurement of the activity. The activity was determined by adding 100 µL of 0.010 M nitrophenolacetate (in acetonitrile), and monitoring the change in absorbance at 410 nm using a HP8452 photodiode array spectrometer. The rate of formation of nitrophenol showed zero order kinetics hence the slope of the absorbance time curve was used as a measure of the activity of the enzyme. The assay procedure for the bead system consisted of adding 0.100 g of beads to a polystyrene centrifuge tube. 25 mL of 0.10 M citric acid/TRIS buffer was added and the system was equilibrated for 10 minutes. 3 mL of nitrophenolacetate was added and 3 mL aliquots were removed periodically to measure the absorbance at 410 nm. The slope of the absorbance vs time profile was used as a measure of the activity. The beads were washed well with fresh buffer to remove the nitrophenolacetate and 25 mL of test sample was added to the tube and equilibrated for 10 minutes. The sample was decanted from the beads which were then washed well with fresh citric acid/TRIS buffer and nitrophenolacetate added to measure the activity. Finally the beads were equilibrated with 25 mL of 1.0 mM zinc in 0.10 M citric acid/TRIS buffer for 10 minutes. The activity was then measured using nitrophenolacetate. The three activity measurements correspond to blank, sample, and fully activated enzyme levels.

RESULTS AND DISCUSSION

An explicit assumption in the enzyme monitor method is that the only species that can react with the apoenzyme is the free ion. This assumption was checked using metal ion buffers prepared using citric acid, NTA, and dipicolinic acid. The results for the citric acid metal ion buffers are shown in Figure 1 for different equilibration times. The metal ion buffers were prepared using a fixed total zinc concentration of 0.10 mM and total citric acid concentrations from 1.0 mM to 0.25 M. The pH was bufferred to 8 using 0.10 M TRIS. The advantage of this buffer system is that the zinc speciation was fixed to give a constant concentration of zinc citrate (0.10 mM) but the free zinc concentration varied from 0.01 to 2 µM. The curves shown in Figure 1 indicate that the rate of enzyme activation varies as the citric acid concentration varies, that is the rate depends on the free zinc concentration and not on the total concentration of zinc or zinc citrate. A similar set of results were found for zinc metal ion buffers using NTA as the complexing agent.

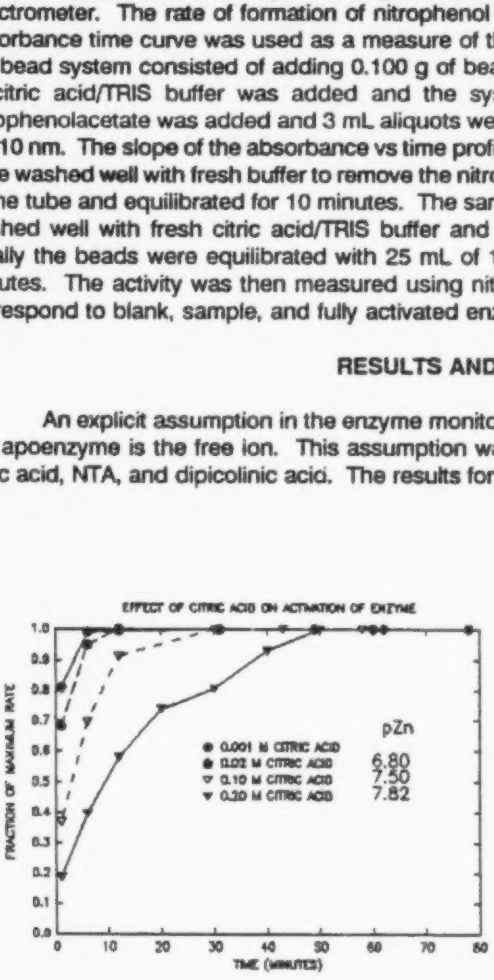


FIGURE 1

The effect of dipicolinic acid/zinc metal ion buffers on the activation of the apoenzyme is shown in Figure 2. These metal ion buffers were made in a similar fashion as the citric acid metal ion buffers, that is a constant total zinc concentration (0.10 mM) and varied dipicolinic acid concentration. The stability constant for the zinc dipicolinic species is much greater than the corresponding citric acid complex. A comparison of the curves in Figure 1 and Figure 2 for similar free zinc concentrations indicates that the enzyme activates much faster for the zinc dipicolinic acid system than for the zinc citric acid system. This indicates that the enzyme is also activated through a reaction between the active site and a zinc complex. A comparison of the activation rate and the solution speciation indicated that it was the zinc complex with one dipicolinic acid ligand (ie ZnL) and not the zinc complex with two dipicolinic acids (ie ZnL_2) that activates the enzyme. This behaviour could be expected because the monodipicolinic acid complex of zinc would be planar and have similar dimensions as the substrate p-nitrophenolacetate.

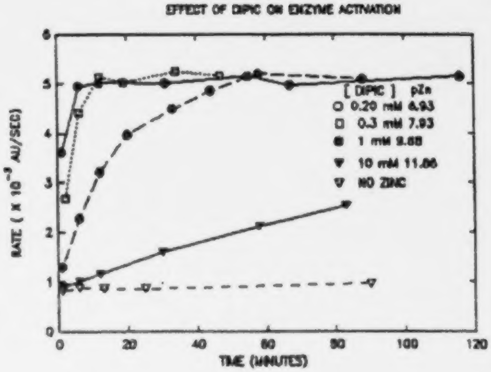


FIGURE 2

The stereochemical selectivity of the apoenzyme is not perfect but the activation of the enzyme can be attributed largely to the free ion and a few select planar complexes. The calibration curves, that is the activity vs pZn profile, for 10 minute and 70 minute equilibration times are shown in Figure 3. The metal ion buffers for these curves were made using either citric acid or NTA. The curves do indicate that the enzyme assay method would be sensitivie to free zinc levels over the range of pZn 10 to 8 depending on the equilibration time.

One advantage to the enzyme method will be the ability to monitor both activation by zinc and inhibition by other metal ions. The effect of copper on the enzyme is shown in Figure 4. This experiment was done by equilibrating the apoenzyme with copper/citric acid/TRIS metal ion buffers for 60 minutes then adding sufficient zinc to make the buffer 1 mM in zinc. The experiment can be done this way because the reverse rate constant for dissociation of the metal enzyme complex (ie k_{-2} in Scheme 1) is very small. It has been noted (Henkens and Sturtevant (1968)), for example, that no exchange of $^{65}Zn^{2+}$ with native zinc was observed over 32 days. The apoenzyme is very sensitive to the free copper ion and it should be possible to extend these studies to the study of other metal ions.

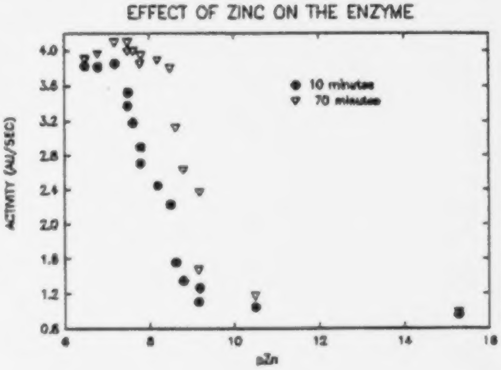
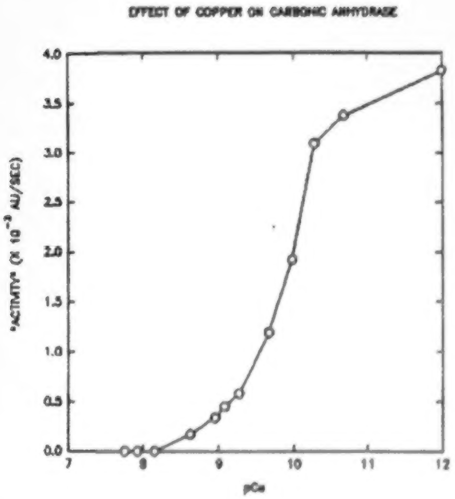


FIGURE 3

There are a number of disadvantages associated with the above enzyme procedure. These include the cost of the enzyme, the time associated with the production of the apoenzyme, the necessity of equilibrating the enzyme and measuring the activity at the same pH, and the necessity of having a sample that does not interfere with the spectrophotometric determination of the activity. These difficulties can be readily overcome by fixing the enzyme to a solid substrate. The enzyme fixed to the controlled glass beads can be reused, the



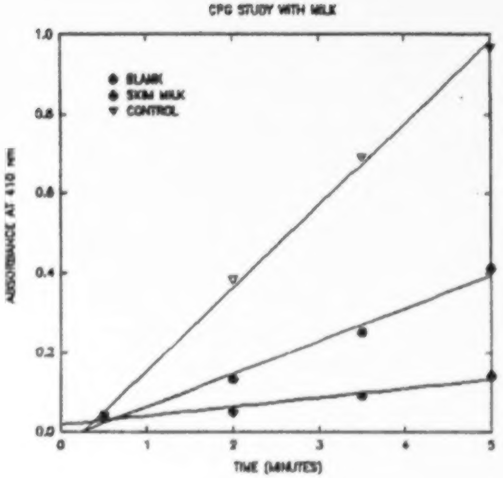


FIGURE 4 **FIGURE 5**

production of the apoenzyme can be done in a batch process, and the equilibrated beads can be separated from the test medium for the assay. Studies of the enzyme fixed to the beads indicated that the activation curve for the system was the same as that shown in Figure 3 and the procedure described in the experimental provides a blank, sample, and control measurement for every aliquot of beads. An example of a sample that would not be amenable to the homogeneous method would be a turbid colloidal suspension. A good example of such a system would be skim milk. This type of sample is considerably more difficult than any natural water sample and typical results are shown in Figure 5. The total zinc concentration in the milk sample was 53.5 μM and the curves in Figure 5 indicate that the free zinc corresponded to a pZn of 8.4.

CONCLUSION

Enzyme assays using carbonic anhydrase or similar metalloenzymes may serve as a convenient monitor of metal availability in aqueous samples. The enzyme fixed to a solid substrate provides a reusable form of the enzyme that may be suitable for multication avialability studies if one complements the enzyme assay with a measurement of the total metal content of the equilibrated enzyme.

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Estimating Metal Bioavailability using Bioassay Techniques

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Bioassays are essential in any study of metal availability because, regardless of the techniques used to measure "available" metal, results must eventually be compared with, or calibrated against, a biological response. The biological response ultimately defines what is meant by the term "available". In addition to this, however, bioassays have also been used to try to quantify the fraction of the metal which is available, or the ability of the medium to chelate or reduce availability, by using a variety of manipulations, often involving a repetition of the bioassay after addition of a standard complexing agent. One approach is to destroy all organic complexing agents present (e.g. by U.V. photo-oxidation) and then add varying amounts of synthetic complexing agents until the same biological response is obtained (e.g. Whitfield and Lewis 1976). This provides a measure of the complexing capacity of the medium, but not a true quantification of the availability of the metal.

A more sophisticated approach to the use of bioassays in quantifying metal availability was developed after it appeared that metal toxicity was often directly correlated to free metal ion concentrations, as measured using metal ion electrodes, or as calculated in defined media using metal speciation models. For example, addition of Tris (tris-hydroxymethyl-aminomethane) can shift the copper toxicity curve for copepods in fresh water to 500 fold higher total copper concentrations. However, the toxicity curves based on free copper ions lie virtually on top of one another, independent of the concentration of Tris present (Borgmann and Ralph 1984). Sunda et al. (1978) showed the same phenomena for cadmium toxicity to grass shrimp in the presence of NTA (nitrilotriacetic acid) or at various salinities. Similarly, it is possible to rationalize much of the variation in the literature on copper toxicity to fish in freshwater by taking pH and hardness into account and expressing toxicity on a free metal ion basis (Borgmann 1983). These, and other, studies suggest, therefore, that in many cases toxicity is directly related to free metal ion concentrations. Unfortunately, metal ion electrodes are often not sensitive enough to measure free metal ion concentrations in natural waters. However, the correlation between metal toxicity and free metal ion concentrations suggest that bioassays could be used to estimate free metal ion concentrations in natural waters. For example, Sunda and Gillespie (1979) removed natural chelators in seawater by ultraviolet radiation, replaced these with know concentrations of NTA, and compared inhibition of glucose uptake in bacteria with free copper ion concentrations calculated from speciation models. This provided a standard curve for predicting free copper ion concentrations in natural waters to which copper had been added.

A somewhat better approach, is not to try to develop a complete speciation model, but to compare toxicity curves in the same water before and after addition of a complexing agent with a known complexing capacity. By assuming that the shift in the toxicity curve represents the concentration of the complex between the metal and the added ligand, the free metal ion concentration can be calculated if the stability constant for the ligand is known (e.g. Borgmann and Ralph 1983, 1984, Sunda and Ferguson 1983).

Unfortunately, metal toxicity is not always a function of free metal ion concentrations alone. For example, copper-amino acid complexes appear to be toxic to Daphnia magna (Borgmann and Ralph 1984) and freshwater shrimp (Daly et al. 1990), and copper-Tris and copper-citric acid complexes have been reported to be toxic to algae (Guy and Kean 1980). Tolerance to free copper ions by cladocera in natural waters is also sometimes lower than in artificial media or well water (Giesy et al. 1983, Borgmann and Charlton 1984), suggesting that copper complexes of some naturally occurring ligands are also toxic. Similarly, free cadmium ion concentrations are not always a good predictor of toxicity to cladocera and fish (Giesy et al. 1977).

One of the reasons that metal toxicity is not always directly related to free metal ion concentrations in natural waters could be that these waters contain ionophores, metal chelating agents which increase the rate of metal uptake by facilitating cation transport across membranes (Levinson et al. 1979). Some examples of ionophores are the lipid soluble complexing agents diethyldithiocarbamate (DDC) and 8-hydroxyquinoline (8HQ). Such complexing agents increase both adsorption and toxicity of copper to algae (Florence et al. 1983). DDC increases accumulation of cadmium by Daphnia, while other complexing agents such as humic acid, EDTA (ethylenediametetraacetic acid) and NTA decrease it (Poldoski 1979). While the water soluble ligands NTA, tannic acid and 8-hydroxyquinoline-5-sulphonic acid decrease copper toxicity to the amphipod Allorchestes compressa, lipid soluble ligands such as 8HQ increase copper toxicity (Ahsanullah and Florence 1984). Since ionophores are complexing agents, their presence decreases free metal ion concentrations, while at the same time increasing metal uptake and toxicity. It is impossible, therefore, to relate toxicity to free metal ion concentrations alone if ionophores are present.

It is still possible to use bioassays to predict free metal ion concentrations in the presence of ionophores or other substances which increase toxicity on a free metal basis (Borgmann and Ralph 1983). Addition of a reference complexing agent still decreases toxicity, but its effectiveness is reduced. The toxicity curve is shifted less towards higher total metal concentrations, and the predicted free metal ion concentration is reduced. However, if toxicity is not solely a function of free metal ion concentrations, then water quality objectives or standards cannot be based entirely on free metal concentrations, and the value of determining free metal concentrations by bioassay, or any other technique, on a routine basis is questionable.

While estimation of free metal ion concentrations is not the solution to defining metal availability, the above studies do suggest an important alternative. Conditions which affect toxicity, such as the presence of ionophores or water soluble ligands, generally also affect uptake. It should be possible, therefore, to obtain a better estimation of "availability" as defined by biological effect, by relating this effect to metal uptake. In fact, several authors (e.g. Davies 1978, Connolly 1985) have suggested that metal toxicity should be expressed relative to the amount of metal accumulated, rather than metal concentrations in the water. Not only does this have the potential to reduce some of the variability in metal toxicity measurements, but it would allow evaluation and interpretation of the large data base in the literature on metal concentrations in aquatic biota. Unfortunately, most experimental studies on metal toxicity, especially with invertebrates and algae, have either related toxicity only to metal concentrations in water, or have not conducted uptake and toxicity tests simultaneously to allow comparison of toxicity with uptake directly.

One of the few studies on the relationship between uptake and toxicity was done with Daphnia. Winner and Gauss (1986) concluded that there was a poor relationship between bioavailability, bioaccumulation and toxicity of copper, cadmium and zinc in multicellular animals because of complex storage, transformation and excretion processes. However, they examined only the effects of hardness and humic acid additions, and their highest humic acid concentration was only 1.5 mg/L. Furthermore, they conducted copper and cadmium toxicity tests using Daphnia pulex and measured uptake in D. magna, because of its larger body size. Consequently, we decided it was time to look at the relationship between uptake and toxicity in a little more detail.

Our experiments were done using the amphipod Hyalella azteca. This species is one of the most sensitive organisms to cadmium toxicity, and chronic toxicity can be determined from survival after 4 to 6 weeks exposure of young (0-1 wk old) amphipods (Borgmann et al 1989). Growth and reproduction are not affected at concentrations below those which cause mortality within 4-6 weeks. Chronic toxicity is strongly reduced by the addition of 20 mg/L humic acid, 0.5 uM EDTA or 10% (by volume) of Hamilton Harbour sediments to Lake Ontario water (hardness 130 mg/L; Borgmann et al. 1990). Addition of 90% distilled water, which reduces the hardness to 10% of Lake Ontario water, has a minor effect on toxicity. Cadmium accumulation was also strongly reduced by addition of humic acid, EDTA, or sediments. The net result was that toxicity was much more constant when related to the amount of cadmium accumulated, rather than the amount of cadmium added, or cadmium measured in the water. The relationship between toxicity and accumulation, however, was not perfect. Decreasing the hardness had little effect on toxicity of cadmium in water, but roughly doubled the cadmium bioaccumulated at concentrations resulting in 50% mortality (the EC50). Addition of sediments increased the cadmium accumulation at the EC50 by about 3 fold. This compares, however, with a 35 fold range in EC50s based on cadmium measured in water, and a 2600 fold range in EC50s based on total cadmium added (Borgmann et al. 1990).

It is not surprising that the relationship between toxicity and bioaccumulation is not always exactly constant. The total amount of a metal accumulated includes metal accumulated at the biologically active site, metal accumulated internally in tissues which are not sensitive to the metal, and metal adsorbed to the exterior of the animal. If the ratios of these metal concentrations do not remain constant, then the relationship between toxicity and bioaccumulation will not be exact. The relationship between toxicity and bioaccumulation will, therefore be a function of factors which affect the internal distribution and adsorption of the metal. However, the relationship between toxicity and metal concentrations in water will be a function of both these factors, and factors which affect the bioaccumulation of the metal. It would seem logical, therefore, that metal toxicity will, in general, be more accurately predicted from metal accumulated than metal in the water.

One of the factors which may influence the relationship between toxicity and bioaccumulation, the fraction of the metal adsorbed onto the outside of the animal, is a function of body size. Smaller animals have a much larger surface to volume ratio, and therefore can be expected to have a greater percentage of the "bioaccumulated" metal adsorbed to the surface. Assuming that the active site of toxic action, at least for chronic toxicity, is not on the surface, this suggests that bioaccumulation in larger organisms might provide a better predictor of effect than accumulation in small animals. Ultimately, the best relationship between toxicity and accumulation should be obtained by relating toxicity to uptake in the target tissue. For example, methyl mercury residues in several tissues of brook trout at time of death were variable for a number of tissues. However, residues in the brain varied very little (McKim et al. 1976), suggesting that this might be the site of toxic action.

Bioaccumulation will not provide a measure of metal availability for some organisms for metals which are biologically essential and regulated. For example, zinc concentrations in the littoral prawn Palaemon elegans are constant up to 316 ug/L in water, and copper uptake is independent of water concentrations up to 100 ug/L. Regulation breaks down at higher concentrations (Rainbow and White 1989). Accumulation will, therefore, only be a useful indication of metal availability at higher concentrations. However, in other species, such as the sublittoral prawn Pandalus montagui, amphipods and barnacles, metal regulation breaks down at much lower metal concentrations (Nugegoda and Rainbow 1988, Rainbow and White 1989). Estimating bioavailability of copper and zinc from bioaccumulation data will, therefore, be restricted to animals with poor regulatory capabilities.

Summary

Bioassays can be used to estimate free metal ion concentrations corresponding to biological effects, but metal toxicity in not purely a function of free metal concentrations. Some metal complexes are readily accumulated and highly toxic. Chemical speciation models, or instruments which measure free metal concentrations, cannot, therefore, be expected to provide accurate estimates of metal availability in all media. Measurement of metal bioaccumulation as a predictor of "available" metal, in terms of a biological response, holds promise. However, research is needed to clarify what factors affect the relationship between uptake and toxicity and how these can be controlled, or compensated for. Ultimately, the best estimate of biologically active metal concentrations may be obtained by measuring metal accumulated in the target tissues where toxicity actually occurs. Since toxicity is not always exactly correlated with bioaccumulation, the term "available" must be defined clearly. Available for uptake does not necessarily mean the same as available to create a toxic, or other biological response.

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TROPHIC TRANSFER OF METALS IN AQUATIC SYSTEMS.

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Radiotracer experiments were conducted to examine the accumulation of metals and other elements in marine herbivorous zooplankton, in which the dissolved and particulate (algal food) source terms were well characterized. The assimilation efficiency of ingested elements (Ag, Am, C, Cd, Hg, P, Pu, S, Se, and Zn) in four species of marine copepods was directly related to the cytoplasmic content in the food, suggesting that these animals with short gut residence times have developed a gut lining and digestive strategy that provides for assimilation of only soluble material within the food. Assimilation efficiencies ranged from 0.8% for Pu to 97.1% for Se. Class A metals, with a strong propensity for hydrolysis, were found to associate with the algal food supply via colloidal attachment to the cells (i.e., particle-particle interaction), do not penetrate into the cytoplasm of cells, are not assimilated in zooplankton, and have short residence times in surface waters because they are removed by rapidly sinking fecal pellets. Class B and berderline metals are found to varying degrees in algal cytoplasm, do assimilate in animals, and have longer residence times in surface waters.

USES FOR SPECIATION MODELS IN DETERMINING BIOAVAILABILITY OF TRACE ELEMENTS.

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Speciation or partitioning among forms appears to have important influences on the bioavailability of trace elements in solution or associated with sediments. Analytical methods for directly determining the bioavailable forms of most elements are inadequately developed for use in nature, but approaches that employ speciation and/or partitioning models show promise. At present, all such models have important geochemical limitations to their applicability. More importantly, meaningful applications of models are being constrained by the failure to consider multiple modes of exposure and relevant biological processes. This paper will suggest that processes in addition to speciation determine trace element bioavailability and that the most important of these differ among elements, among organisms and with the most important mode of exposure of the organism. If accurate, speciation models may be an important component in determining the bioavailability of an element, but no one modelling approach will provide answers for all organisms or all elements, and in many instances interfaces with models of biological processes will be necessary to obtain meaningful results. Examples are provided from studies of Se bioavailability.

SITE-SPECIFIC ASSESSMENT OF THE AVAILABILITY AND EFFECTS OF METALS IN SEDIMENTS.

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Since aquatic sediments can become contaminated with metals it is important to determine if the presence of these metals is having an adverse effect on benthic invertebrates. To do this, one must be able to measure or predict the activities of mixtures of metals as well as that of the individual dominant elements. Geochemical simulation models attempt to predict the activities of metals from measurements of important controlling factors. Alternatively, operational measurements of metal activities can be made *in situ* or under laboratory conditions. *In situ* measurements include the use of passive samplers known as "peepers". Laboratory techniques include a number of manipulations, which include separations, extractions and fractionations. Bioassays can also be used and manipulated to determine the activity of metals and their relative contribution to the toxicicity of sediments to benthic invertebrates. Here I will discuss the strength and weaknesses of each technique and discuss which techniques hold the most promise for the future and comment on research and development needs.

COMMENTS FROM REPRESENTATIVES OF INDUSTRY AND GOVERNMENTAL AGENCIES RESPONSIBLE FOR ENVIRONMENTAL MANAGEMENT.

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Industry, in general, is willing to abide by whatever regulations are put in place regarding the bioavailability of metals in their effluents so long as the rationale for these regulations is reasonable. The regulations and their appropriate rationale are, however, the sole responsibility of the provincial and regulatory agencies and not that of industry. There is an awareness on the part of industrial environmental managers that the "total" or "extractable" metal content of their effluents do not accurately reflect the bioavailability and potential toxicity of these metals. However, industry seems generally unwilling to explore this relationship unless the regulatory monitoring requirements related to the metal content of their effluents becomes a financial burden. It is much easier to let government set the criteria and then challenge the rationale behind the criteria.

From a regulatory perspective, identifying the extractable and total metals in effluents is the "path of least resistance" in the identification of criteria for effluent metal concentrations. There is a realization among regulatory agencies that this may substantially over-estimate the bioavailable metal component, but they currently do not have available a reliable analytical method for quantifying this component. If such an analytical method did exist, there might still be a great reluctance on the part of regulatory agencies to use it. The use of a criterion related to the bioavailability of metals might, in some cases, allow industry to discharge more metals than would be permitted under a total or extractable metal based criterion. There are very few regulatory agencies which want to proceed in this direction.

WORKSHOP

MISA Biomonitoring in Ontario

Chairs: T. Kierstead and G. Westlake

THE APPLICATION OF TOXICITY TO MISA EFFLUENT LIMITS REGULATIONS

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ABSTRACT

We tend to take a broader view of effluent toxicity than do our engineering colleagues. Though much of our work involves acute lethality, our view includes chronic and sublethal effects as well as contamination of biota. Toxicity is not just another parameter to be monitored and controlled to meet our objective - its control, with few exceptions, is our objective. The implementation of effective strategies for monitoring and controlling effluent toxicity has required difficult and practical choices to be made in Ontario. Although we still have occasion to have to defend the idea, clearly chemical characterization is no substitute for whole effluent toxicity testing. Ideally we should be monitoring and controlling all possible effects of discharges on all organisms of the aquatic community. However, since we are regulating all major industries in the province at the same time, Ontario chose to monitor with rainbow trout and *Daphnia magna*. And, since it is not feasible to monitor these samples with chronic or sublethal tests, Ontario has decided to use acute lethality tests as a first line of defense. Eliminating the acute lethal effects effluents in the province at the point of discharge will be an important achievement. Usually, we will be reducing or even eliminating measurable sublethal and chronic impacts at the same time.

SUMMARY

The objective of water pollution control is to reduce or eliminate harmful effects on aquatic life and to reduce or eliminate the contamination of aquatic life and drinking water and to reduce unnatural rates of eutrophication. In the broadest sense, whether it be for MISA (Ontario's Municipal/Industrial Strategy for Abatement), for Canadian federal regulations, or for USEPA; whether it be for Best Available Technology or water quality-based strategies, all water pollution control relates to toxicity. This broader view of toxicity is not always shared by our engineering colleagues. Any discharge that causes harmful effects on aquatic life is by definition **toxic**. Contamination of aquatic life is a concern if, it reaches **toxic** levels in the organisms themselves or it causes **toxic** effects in humans or wildlife that consume them. Contamination of drinking water is a concern because it may be **toxic** to humans or livestock. Therefore, toxicity is not just another parameter to be monitored and controlled to meet our objective - its control is our primary objective. All that is left after you eliminate toxicity driven aspects of water pollution control are the aesthetic or eutrophication problems and the political decisions, as well as the economic realities that make these decisions necessary.

Imagine that you are an extraterrestrial being arriving on our planet for the first time and that you encounter the parts of a dismantled clock. Could you grasp the significance of this pile of objects? Even if you figured it out and put it back together, would you expect to be able to set the time back to where it was before the clock was taken apart? Obviously, it would be easier to observe the clock functioning and then take it apart to find out what makes it tick. Similarly, we will have a better chance of understanding the impacts of effluents by first observing their effects on aquatic life as intact, whole effluents before we study their components. Whole effluent toxicity testing comes closest to doing this for us.

I sometimes get the impression that many people hold a vision of the ideal future for aquatic environments as clean, clear water inhabited only by uncontaminated, edible, non-threatened fish. We all know, or should, that this is unrealistic since these fish need something to eat, and something to recycle their carcases when they die, and something to compete with them so that the world does not become piled high with fish. In short, they exist in an aquatic community all of which must be protected if any of it is to survive.

Ideally, therefore, we should be monitoring and controlling the effects of discharges on all organisms of the aquatic community. Practically, this is impossible since we are regulating all discharges in the province at the same time, so we have had to set priorities. The first priority chosen by Ontario has been fish. Fish survival must be considered important because it protects water uses of considerable commercial, recreational and aesthetic value. Fish populations take a very long time to adapt to alterations in their environment and many locales in Ontario are inhabited by rare sub-species that cannot be replaced. The second priority chosen by Ontario were crustaceans. These, represented by *Daphnia*, are sensitive to different substances than are fish. They form large important part of the aquatic ecosystem. We also have well established laboratory procedures using them.

Some people do not understand why we should control toxicity for more than one organism. Not doing so would ignore the fact that life varies in its sensitivity to toxic substances. If this were not so, pesticides would kill both the farmer and his crops during spraying. Similarly, for some effluent samples *Daphnia* are more sensitive than are trout whereas, for other effluents the reverse is true. Also, effluents are complex changing mixtures. At one time, a given effluent might kill more trout while at another time, the same effluent might kill more *Daphnia*. Both should be monitored and controlled independently. Industry is understandably nervous about how many other forms of aquatic life we could use. But, I do not think we can or should reassure them that we will forever stay with these two because our responsibility is to protect all forms of life.

The recommendation has been made that we monitor *Daphnia* toxicity but only use the fish test for limits. This could be a very hazardous approach. What happens when we find an effluent that kills *Daphnia* but not trout? Such effluents exist and they are among the largest flows in the province. Any effluent could have this characteristic at one time or another. A government agency cannot fail to act on the knowledge that discharges could kill such an important animal.

Based on the above and other considerations the Ontario Ministry of the Environment has presented to industry, the following position regarding the incorporation of toxicity into new effluent limits regulations. The effluent limits regulations will be written to require that:

1. all dischargers monitor effluents for acute lethality to fish and to *Daphnia* according to MOE protocols.
2. all effluent samples shall not be acutely lethal to fish or to *Daphnia*.

3. the criterion for both tests is 50% survival after exposure to 100% effluent; that is, if an effluent kills no more than 50% of the animals exposed to the undiluted effluent, it passes the test.

4. effluents whose samples pass, may be monitored with a single concentration test of undiluted effluent.

5. Dischargers shall monitor the toxicity of their effluents for a year. The Monitoring Regulation data will usually satisfy this requirement. For effluents that have passed for this period, testing will be continued at a frequency of four times per year. Until the discharger meets this condition, we will require monthly testing. For effluents that fail either test, the discharger will be required to sample weekly for toxicity and to report on the probable causes. Monthly sampling will resume if the samples pass for 3 consecutive weeks.

6. If an effluent fails for either or both test organisms on any three of the weekly samples, the Ministry will require the discharger to develop and perform a Toxicity Identification/Reduction Evaluation (TI/RE).

7. enforcement actions may result if any sample fails either the trout test criteria or the *Daphnia* test criteria at any time or if the discharger does not perform a TI/RE as required.

8. the Effluent Limits Regulations will be at least as stringent as toxicity requirements of federal regulations and that they are compatible with existing international accords and agreements.

9. sublethal and chronic toxicity monitoring for assessment will be performed twice per year on discharges that are consistently non-lethal by the compliance date to both animals.

The focus, as first line of defense, on acute lethality tests using two aquatic animals was a practical choice. Unless we were prepared to be highly selective about both frequency of sampling and which discharges to monitor, we could not have completed the monitoring, auditing, and data management. I am not convinced that we would have gained significantly from the use of sublethal or chronic tests in this context. First, effluent toxicity is highly variable over time. This variability is frequently greater than the difference between acute and chronic responses. Secondly, for complex effluent mixtures, acute to chronic ratios are often small and the increased sensitivity of chronic or sublethal results does not justify the greatly increased effort required to do them. Thirdly, the more complex the test procedure is, the more difficult it is to obtain consensus on formal test protocols and the more prone to alternate interpretations the results are.

Where the mode of toxic action is similar for acute, chronic and sublethal impacts, elimination of acute lethality from all effluents at the point of discharge is the most effective strategy for controlling these as well. The proposed effluent limits regulations, however, include a monitoring requirement for sublethal and chronic toxicity monitoring for those effluents that meet the acute lethality criteria.

ONTARIO REFINING INDUSTRY EXPERIENCE WITH TOXICITY AND MISA. J.T.Kierstead, NOVA Petrochemicals Inc., Corunna Operations, Box 3060, Sarnia, Ontario, Canada (519 337 6484).

Ontario Refineries have steadily reduced the amount of contaminants in their effluents. Rainbow trout acute lethality testing has been routinely used since the early 70's. The use of *Daphnia magna* acute lethality (and a new protocol) in the MISA monitoring regulation however, was a new experience for refiners, and one which raised concern about its relevance and meaning. MISA is an end-of-pipe, technology driven regulation which will result in effluent limits for fish and *Daphnia*. Will this protect the receiving environment? Is this a practical approach? This paper will examine the refining industry experience with the monitoring regulation and make comments on the strengths and weaknesses of the soon to be introduced limits regulation.

OPPORTUNITIES, DIFFICULTIES AND CHALLENGES FOR ENVIRONMENTAL TESTING LABORATORIES.

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The volume of toxicological tests has grown rapidly as a result of the introduction of MISA. For private laboratories, this has presented opportunities to diversify activities. MISA poses a number of challenges, including the response to a high demand and quick turnaround of test results, so that delays are not encountered by Government in the administration of the program.

One concern facing private laboratories is the direction of the future needs of Government and Industry. Consultation by government and industry with the consulting sector in the early developmental stages of programs requiring expansion beyond existing capacity in the sector will facilitate the efficiency and economy of supply of services. In order to ensure that a healthy service sector is in place to meet future needs, Government needs to continue to support research and development. It should also be the role of Government to implement programs for inspection and quality evaluation of specific laboratory services as they apply to government regulatory programs to ensure quality of the data being produced.

FEDERAL INVOLVEMENT IN THE ONTARIO MISA PROGRAM.

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In 1985, Environment Canada accepted an invitation from the Ontario Ministry of the Environment to co-operate in the development of the MISA program. By adding its technical and scientific expertise to the program, Environment Canada is building on previous federal-provincial co-operative efforts to achieve the goal of virtually eliminating all toxic discharges to the Great Lakes. MISA principles such water quality protection, use of best available technology economically achievable (BATEA) and pollution control at source are fully supported by Environment Canada. In the area of aquatic toxicology, Environment Canada technical staff have been a partner with Ontario in developing their effluent toxicity monitoring and control requirements since 1985. Specific examples of Federal support in the development of MISA toxicity requirements and Ontario involvement in the development of new Environment Canada effluent regulations and biological testing procedures will be outlined.

WORKSHOP
Marine Environmental Quality Monitoring

Chairs: M. Pomeroy and M. Waldichuk

DESIGN OF THE GULF OF MAINE MARINE ENVIRONMENTAL QUALITY MONITORING PROGRAM

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Under the direction of the five jurisdiction Gulf of Maine Council on the Marine Environment, a monitoring program was designed to meet three goals: provide information on the status, trends and sources of human health risks; provide information on the status, trends and sources of risks to ecosystem integrity; and provide this information in an appropriate and timely manner to managers. The program is designed to integrate and enhance numerous existing municipal, state, provincial and federal programs, including the NOAA Status and Trends Program, the FDA National Shellfish Sanitation Program, and the CWS Seabirds Contaminants Program in addition to existing effluent permitting programs. Information will be integrated through use of a personal computer-based link designed to use the existing data base management system in those agencies. Information interpretation will be completed by the Council's Working Group. Given that one of the options is accepted by the Council, a pilot project will be implemented as early as 1991.

SUMMARY

The Gulf of Maine is no longer pristine. Tons of raw and partially treated sewage are discharged into the Gulf each day. Industrial discharges and urban and agricultural runoff introduce toxic contaminants and pathogens to marine and estuarine waters on a chronic, and at times, acute, basis. Increased fishing effort has reduces fish stocks to all time lows. Coastal development has encroached on environmentally significant marine wetlands. Accidental spills of oils and other toxic material place additional stresses upon the Gulf environment.

To understand and manage the impact of such stresses on the health of the Gulf ecosystem requires accurate understanding of the nature, scale, and impact of environmental perturbations in the Gulf. As a step toward generating the requisite information, the Gulf of Maine Council on the Marine Environment has established a tightly focused and pragmatic environmental quality monitoring plan for the Gulf of Maine.

The Gulf of Maine council on the Marine Environment was established by the Governors of Maine, Massachusetts and New Hampshire, and the Premiers of Nova Scotia and New Brunswick to improve the environmental management of the Gulf of Maine.

The Council has identified assessment of the health of the Gulf as of pressing importance. The council initiated development of a monitoring plan as a first step toward improving environmental management of the Gulf, envisioning a program that will allow evaluation of environmental quality of the Gulf while improving the effectiveness of prevention and remediation efforts.

The monitoring plan is based on a mission statement provided by the Council:

> It is the mission of the Gulf of Maine marine environmental quality monitoring program to provide environmental and resource managers with information to support sustainable use of the Gulf, and allow assessment and management of risk to public and environmental health from current and potential threats.

The Council charged the Gulf of Maine Working Group, the management component of the council, and its monitoring sub committee with identifying the environmental quality issues of greatest importance to the Gulf States and Provinces and with developing a monitoring plan to address these issues.

As part of this process, at the December 1989 Conference on Sustaining the Common Heritage of the Gulf of Maine, working sessions were devoted to monitoring and a workshop was held in Halifax in early June, 1990 to review a draft report on the proposed monitoring program. Scientists, environmental managers and policy-makers from throughout the Gulf region worked together to develop consensus on goals and objectives and to begin the process of identifying priorities and selecting appropriate monitoring methodologies. The current plan reflects the results of these consultations.

Three monitoring goals were established to meet the Program's Mission:

- To provide information on the status, trends and sources of risks to the marine environment in the Gulf of Maine;
- To provide information on status, trends, and sources of marine-based human health risks in the Gulf of Maine; and
- To provide appropriate and timely information to environmental and resource managers that will allow both efficient and effective management action and evaluation of such action.

Several objectives were developed to meet these goals and are listed in the planning document. To narrow the scope of the monitoring plan the objectives were ranked in order of importance; the plan was developed to address only the top three objectives. The remaining objectives will be addressed as the program is implemented and resources are available. In order to rank the priority of the monitoring goals and objectives, a survey was distributed at the December 1989 Conference and mailed to over 150 scientists, environmental managers, policy makers, and others in the Gulf. The rankings were further discussed at the workshop in Halifax. The three objectives with the highest priority were:

1. To assess the status and trends in the marine environment by monitoring appropriate indicators, especially those that will allow early identification of change in environmental quality:

2. To assses the existing levels, the trends, sources, and economic impacts of acute and chronic risks to human health from toxic compounds transmitted through marine foods and water contact; and

3. In cases where environmental degradation is suspected, to identify the probable causes, especially as they reflect anthropogenic impacts and cumulative effects.

The participants of the workshop identified an ancillary priority that is not related specifically to monitoring, but should be an important part of any environmental management strategy for the Gulf: all existing environmental data on the Gulf must be organized, assessed for quality, and made accessible to a wide range of users.

The monitoring plan described outlines only the monitoring methods needed to meet the three highest priority monitoring objectives. It then identifies ongoing monitoring programs in the Gulf that are addressing these objectives.

The Monitoring Subcommittee of the Working Group intends to establish the broader goals and objectives of a Gulf-wide environmental quality monitoring program as the first step in the development of such a program. Further implementation of a monitoring program will require establishing specific monitoring methodologies, setting acceptable levels of precision and accuracy, and developing detailed sampling designs which specify the number of samples to be collected, the exact locations, and the laboratory procedures to be used for analyzing the samples. These are details best left to practitioners in the field.

Ad hoc committees will be formed to identify specific implementable monitoring methodologies for the priority objectives identifies in the plan. In addition, the plan will be reviewed at a major scientific conference on the Gulf of Maine and at this Symposium.

The monitoring methods presented here are developed from a review of methods currently used in other programs and from a list of monitoring questions that were developed to address each objective. The questions are categorized in terms of six monitoring parameters which include:

1) The variable to be monitored,

2) The sampling medium in which the variable is measured (i.e. soft bottom, hard bottom, tissue etc.),

3) The geographical scale/location where sampling should take place,

4) The frequency with which the variable should be monitored,

5) The field methods to be used to monitor the variable, and

6) The type of data analyses needed to provide the information to answer the monitoring question.

The plan includes identification of the proposed areas where monitoring should take place and the estimated cost of monitoring other appropriate variables. In all cases cost estimates are based on the assumption that the private sector will do the work. It is estimated that monitoring a broad range of indicators to meet the first objective (assessing the marine environment) will cost of $3,000,000 US annually. This assumes that existing monitoring programs can be modified as needed to collect the appropriate date in their current study areas. The estimated cost for collecting the information needed to meet the objective with the second highest priority (assessing human health risk cost estimate, however, is based on only monitoring the risks of mercury and PCB's, the two toxic compounds for which standards in foods have been developed. There is a major need to fund additional research to understand the human health risks from other toxic compounds. The costs for meeting the objective with the third highest priority, that of identifying causes, cannot be estimated at present because the area and scale of environmental changes have not yet been identified.

The plan also outlines four additional aspects of a monitoring program:

- The procedures to facilitate the transfer of information between the scientists analyzing the monitoring data and the environmental managers who will be using the information to develop management actions,
- Guidelines for developing a database for storing the information collected by the monitoring program, and
- An implementation plan incorporating a pilot program utilizing the "Mussel-watch" concept.

As a strategy for implementation, the plan will build on monitoring activities currently underway in the Gulf. For example, it is anticipated that the Status and Trends Program of the U. S. National Oceanic and Atmospheric Administration will be expanded to answer questions about the health of the larger Gulf ecosystem. Gaps in existing programs will be identified and new programs designed. In addition, the plan will integrate local problems, such as shellfish closures, that occur throughout the Gulf region. Data collected from coastal embayments on toxic contamination, nutrient enrichment, and shellfish and beach closures will be augmented by similar data collected in other industrialized embayments along the Gulf shore. It is our hope that this collective approach will yield better solutions to problems encountered or anticipated in such areas.

The success of this endeavour will depend on:

* the cooperation of State, Provinces and federal agencies in adapting existing monitoring programs to serve the objectives of the Gulf program as well as their own objectives;
* funding for new monitoring to fill gaps identified in existing monitoring activities;
* regional coordination to provide guidance for the development and implementation of the program;
* a database management system that will allow information generated by the monitoring program to be readily available to environmental managers throughout the region; and
* links to a geographic information system such as Environment Canada's FMG project.

The Monitoring Subcommittee invites your comments on this monitoring plan. Further development of this plan requires the informed participation of monitoring professionals, other scientists, environmental managers, and policy-makers. Please forward your comments to the Monitoring Subcommittee, c/o Maine State Planning Office, Station 38, Augusta, Maine 04333, so that they may be incorporated in further iterations of the plan.

LESSONS FROM U.S. NATIONAL AND REGIONAL MONITORING PROGRAMS.

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Long-term inconsistency and poor availability of data and supporting information, are two major problems inhibiting successful assessment of long-term pollution trends in coastal waters of the United States. Large-scale geographical and long-term trends of contaminant concentrations in fish, shellfish, and sediments were developed from recent and historical data collected from a dozen national and several hundred regional and state monitoring programs conducted aperiodically over the past 25 to 30 years. National monitoring programs, involving up to 200 sites, were underway between 1965 and 1972, 1976-78, and 1985-present under various agency sponsorship. State and regional monitoring programs complimented federal programs in some areas (Maryland, Texas, California) but other areas were neglected by both national and state agencies. Reconstructions revealed 100-fold declines of DDT and other pesticides on a national basis during the past 20 years, while PCB concentrations declined only near well-known major point sources. Regional analyses revealed no substantial changes in concentrations of most metals in biota, despite marked declines in inputs and sediment concentrations supporting the idea that metals have not been important contaminants of the sea coast.

Once a major monitoring program begins, all effort should be made to continue it and to resist change even in the face of changing technology. To date, there remains no national or regional commitment to establishing, on a continuing basis, data management systems that accommodate historical as well as current data and supporting information. It is not necessary that all such data be digitized--availability alone is half the battle!

A STUDY OF BENTHIC CONTAMINANTS IN VANCOUVER HARBOUR, B.C. TO ASSESS THE ENVIRONMENTAL QUALITY.

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In May of 1985, Environment Canada initiated a study to assess the benthic environmental quality of Vancouver Harbour, B.C. The main study objectives included the distribution of selected contaminants of the sediments and bottom-dwelling biota in the harbour; the identification of potential sources of urban and industrial contaminants; and the need for remedial measures to improve the environmental quality. A total of 88 stations for sediment and 11 trawl stations for biota have been sampled in Vancouver Harbour (from Point Atkinson to Port Moody Arm) from May 1985 to October 1989. Arsenic, cadmium, copper, chromium, iron, mercury, nickel, lead, zinc, and polycyclic aromatic hydrocarbons were among the chemical parameters quantified in the sediments and biota. Additional chemical parameters measured in the sediment included hydrocarbons, chlorophenols, and polychlorinated biphenyls (PCB). Further investigations looked at the prevelance of liver lesions in Engish sole in association with chemical exposure.

POSTER SESSIONS

EXTENDED ABSTRACT

INTER-SPECIES ACUTE TOXICITY CORRELATIONS OF 267 CHEMICALS

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SUMMARY

The acute toxicities of 267 compounds for six aquatic and one terrestrial species were investigated with correlation, principal component and cluster analysis techniques for relationships with each other and with the compounds' octanol/water partition coefficient. Selection of the investigated chemicals was based on the availability of at least three of the following measured parameters: Acute (24-hr to 96-hr) lethal concentrations (LC50) to the fish fathead minnow (Pimephales promelas), the fish goldorfe (Leuciscus idus melanotus), the zooplankter Daphnia magna, the ciliate Tetrahymena pyriformis, the algae Scenedesmus quadricauda, the (30-min) inhibitory concentrations (EC50) to the luminescent marine bacterium Photobacterium phosphoreum (the Microtox™ test), the acute oral dose (LD50) for the common rat, and the octanol/water partition coefficient (logP or log K_{ow}).

The results indicate highly significant correlations between the fathead minnow, goldorfe, Tetrahymena, Daphnia LC50 and the Photobacterium EC50 concentrations. The cluster and principal components analyses did not detect any clearly defined groups of compounds. The toxicities were also highly collinear with the octanol/water partition coefficients for all species except the rat, where two relationships are indicated, with the division at logP = 2.00.

DATA AND METHODS

Toxicity Data

The toxicity data were mostly obtained from the literature. For the selection of compounds from the data base, the following criterion was applied: At least three measured values of the eight toxicity and physical properties had to be available. This selection resulted in 267 compounds from a variety of chemical classes including aliphatic, aromatic and five- and six-ring nitrogen heterocyclic compounds, with many different substituents and/or functional groups, including nitro, amino, hydroxy, cyano, keto (aldehyde and ketone), chloro, bromo and fluoro groups, for which indicator values are used to show the absence (0) or presence of one (1) to five (5) of each of the groups. All toxicity values are converted to the negative logarithm of the millimolar concentrations.

Abbreviations

MTOX	Microtox™ test (Photobacterium phosphoreum), 30-min EC50, static
FHM	Fathead minnow (Pimephales promelas), 96-hr LC50, flow-through
GO	Goldorfe (Leuciscus idus melanotus), 48-hr LC50, static
DM	zooplankter (Daphnia magna), 24-hr LC50, static
BA	blue alga (Scenedesmus quadricauda), 24-hr LC50, static
TEHY	ciliate (Tetrahymena pyriformis), 48-hr to 60-hr LC50, static
RAT	rat, 96-hr single oral dose LD50

Specifics of Data Set

The total number (n) of compounds measured for each parameter is given as follows:

Parameter	MTOX	FHM	GO	DM	TEHY	RAT	BA	logP
n	249	145	66	108	114	160	56	260

Although measurements were available for at least three parameters for all of the 267 compounds in the data set, the numbers of compounds with measurements on the same three or more parameters was much lower. Table 1 gives an example on the distribution of phenolic compounds.

Table 1. Distribution of compounds containing the phenol functional group and with measured MTOX and logP values.

Functional groups	Number in set[a]
1 phenol group	55
1 phenol group only	22
1 phenol group and ≤1 aromatic ring Cl	39
1 phenol group and ≥2 aromatic ring Cl	16

[a] Exclusive of outlier 4-ethylphenol.

RESULTS

Cluster Analysis

Pairwise plots of the 59 compounds show that all parameters have monotonic relationships, increasing together over the range of observation, with the exception of the pairs, which include rat, for which such a simple relationship does not hold. Five clusters, accounting for 76% of the total variation in this data set, divide the range of each variable into groups with increasing means but not necessarily non-overlapping ranges (Fig. 1). Cluster analysis based on only FHM and DM (73 compounds) accounts for 87% of the total variation and provide five non-overlapping clusters, however, cluster membership is not stable when going from the 59 compounds to the set of 73. Thus, although these clusters account for a reasonable amount of variation, they are neither stable nor separated from each other.

The alcohols span most of the logP range and appear in four of the five clusters, as do the keto compounds. The only group of compounds restricted to one cluster are the compounds with one phenol group, however, this cluster (cluster 3) also contains compounds with three of the other identified functional groups. Thus, the clusters do not contain unique functional groups as the compounds containing a particular functional group span a considerable portion of the logP range.

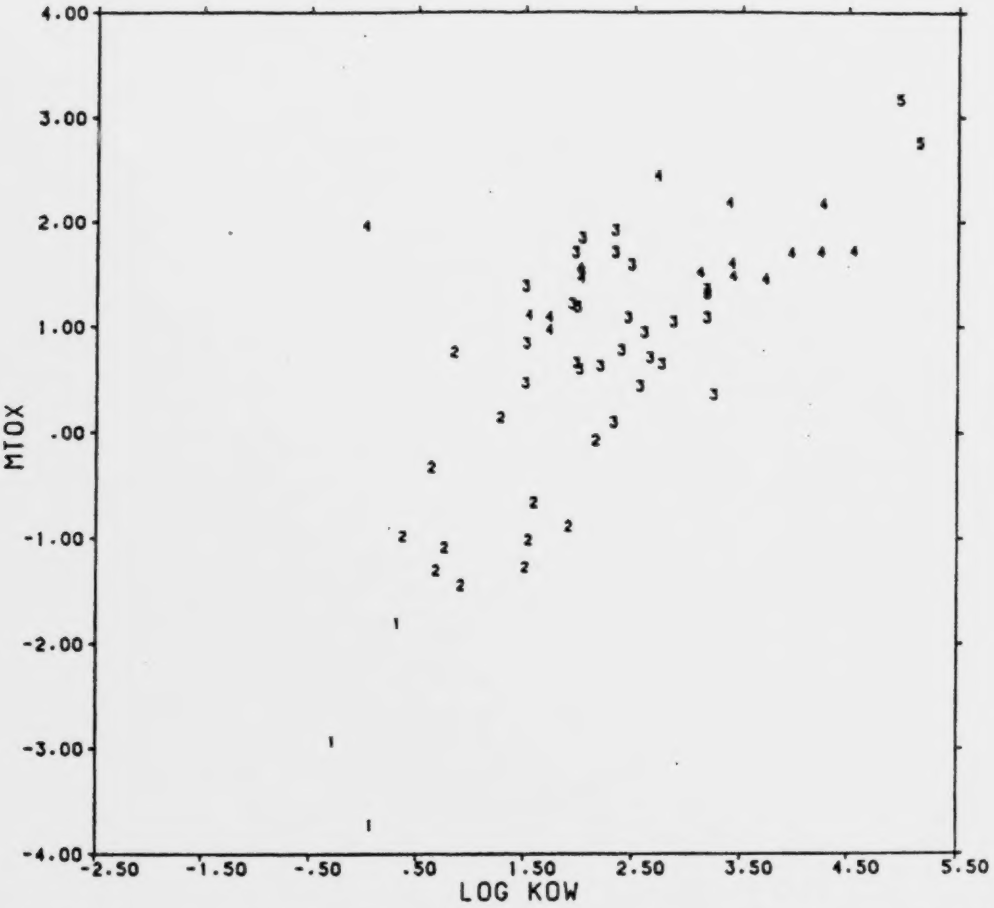

Fig. 1. Five-cluster analysis of MTOX, FHM, DM, RAT and logP subset (n=59) in MTOX/logP plane; (logP = log K_{ow}).

Principal Component Analysis

The first two principal components (PC1, PC2) account for 90% of the variance (Table 2) and reflect the pairwise correlation (Table 3). The plots of the compounds on the first two components, suggest a group of compounds with scores on PC1 greater than 0 and another group with scores less than 0, where the scatter is greater along the PC2 axis in the former and along the PC1 axis in the latter. Two compounds are separated at the top of the plot. However, when the functional groups are identified, again as above, the compounds with different functional groups intermingle. The reduced dimensional plot is dividing the range of the original variables, although at a larger interval, as did the cluster analysis (Fig. 1). This can be seen by identifying the clusters to which compounds were assigned in the cluster analysis on plots of PC1 versus PC2.

Table 2. Principal components of the 59 compound subset

	Standardized Eigenvector				
Parameter	1	2	3	4	5
MTOX	1.00	-0.00	-0.55	-1.00	-0.19
FHM	0.91	-0.20	0.71	0.38	-1.00
DM	0.91	-0.38	-0.84	0.70	0.49
RAT	0.37	-0.59	1.00	-0.29	0.82
logP	0.74	1.00	0.40	0.17	0.47
% variation					
single	78.6	11.6	3.7	3.4	2.7
cumulative	78.6	90.2	93.9	97.3	100.0

Table 3. Correlation matrix

	MTOX	FHM	DM	RAT	logP
MTOX	1	0.85	0.85	0.57	0.71
FHM		1	0.85	0.64	0.66
DM			1	0.62	0.59
RAT				1	0.23

Regression Analysis

The largest subset of data available for pairwise relationships is the set of 242 compounds with MTOX and logP measurements with the features of a dense band of points, the extension of which in the lower MTOX values, suggests curvature of the relationship, and above which are a few scattered compounds with higher MTOX values for the corresponding logP value. The identification of compounds with specific functional groups (Fig. 2) suggests that compounds with different functional groups may have different relationships between MTOX and logP and show that some of the higher MTOX values are atypical of the remainder of compounds with the same functional group present. The difference in the relationship for the sets of compounds where either one phenol or one pyridine (nitrogen containing six-membered ring) or no functional groups are present, is primarily in the slope of the line.

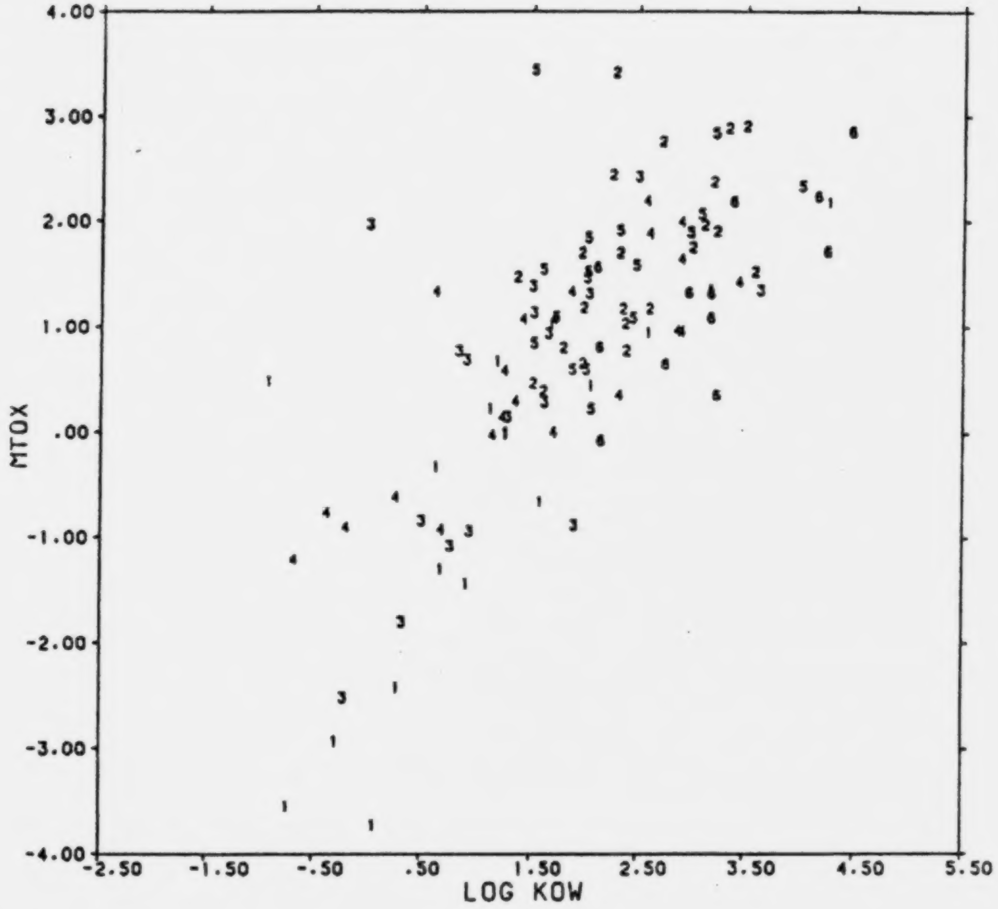

Fig. 2. Plot of MTOX vs. logP (log K_{ow}) by (six) functional groups (n= 109)

For the set of compounds with one keto group, logP is not a good predictor of MTOX. The presence of two or more aromatic chloro substituents when one phenol group is present significantly alters the relationship between MTOX and logP ($p<0.01$) relative to that when fewer than two aromatic chloro groups are present with one phenol group. In fact, at the $p=0.03$ level, for the latter compounds, MTOX is significantly higher than for the former compounds, over the common range of logP observed here.

A relationship between MTOX and logP very different was found for the simple carboxy (alcohol+keto) series. The increase in unit of MTOX per unit change in logP is much greater and the residual variation is very small. Similarly, precisely determined linear relationships between each of FHM, GO, DM, BA with logP were found with r^2 values of 0.984 (n= 17), 0.979 (n= 11), 0.949 (n= 12) and 0.927 (n= 11), respectively. The increase in toxicity with increasing number of carbons in the chain is shown in Fig. 3; excluding c-hexanone, butan-2-ol and propan-2-ol, the correlation coefficient is r^2= 0.985.

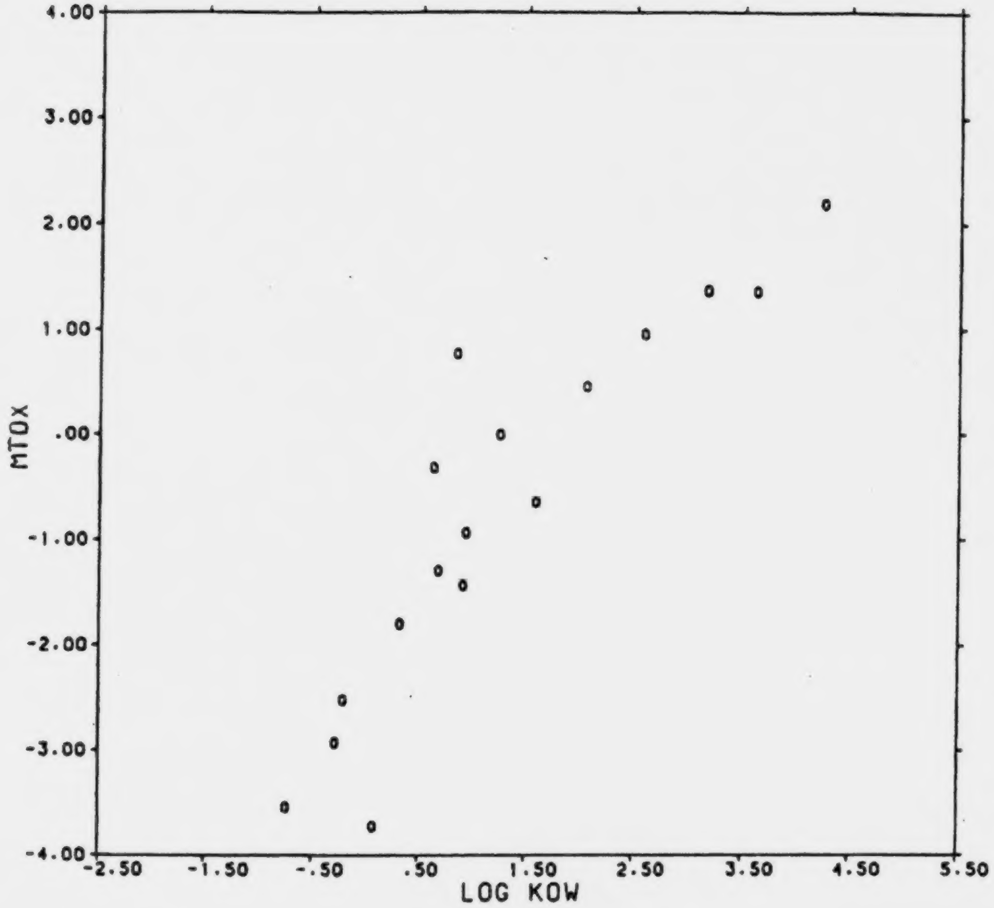

Fig. 3. Plot of MTOX vs. logP (log K_{ow}) for 17 alcohols and ketones

This series of alcohols and ketones are part of a set of 127 compounds for which measurements are available for MTOX, FHM and logP. Although there is considerably more scatter for MTOX, this series tends to form a lower border for the band of points in the plots of FHM versus logP and MTOX versus logP, which has long been recognized as the narcosis cutoff.

The prediction of toxicity from toxicity measurements in organisms of lower order and logP was considered for the 127 compounds with MTOX, FHM and logP values and the 93 compounds with MTOX, FHM, RAT and logP data. For the former set, linear relationships with FHM as the dependent variable were fitted by regression. LogP alone adequately accounted for the variation in FHM for compounds with either one phenol group only or none of the identified functional groups present, but in keeping with the observation made above, logP was a poorer predictor variable than MTOX for compounds with 1 keto group (Table 4). When functional groups not identified, both logP and MTOX were needed as predictor variables.

Table 4. Linear relationships of fathead minnow (FHM), Microtox (MTOX) and octanol/water partition coefficient (logP)

Dependent variable	Predictor variable	Functional group	Number of compounds	r^2
FHM	logP	1 phenol only	15	0.77
FHM	logP	none	9	0.79
FHM	MTOX	1 keto only	11[a,b]	0.58
FHM	logP, MTOX	not identified	126[a]	0.76

[a] Acrolein was excluded.

[b] 2-decanone was excluded because its logP was much higher than others and as a lone point would influence the regression too much.

Plots of the 93 compounds with MTOX, FHM, RAT and logP measured suggest that non-linearity exists in the relationships between RAT and logP and RAT and MTOX. A segmented regression between RAT and logP (Fig. 4) explains nearly as much of the variation in rat toxicity as the regression with MTOX, FHM and logP (Table 5). Since the relationship between RAT and FHM is more linear, FHM also explains nearly as much.

Table 5. Regression relationships of RAT, FHM, MTOX and logP

Dependent variable	Predictor variable(s)	Range of logP	Number of compounds[a]	r^2	Regression	
					Intercept	Slope
RAT	MTOX, FHM, logP	all	91	0.41		
RAT	logP	all	91	0.09	-1.33	0.17
RAT	logP	<2.00	48[b]	0.37	-1.74	0.69
		>2.00	43	0.37	-1.88	0.28
RAT	FHM	all	91	0.34	-1.16	0.36

[a] Acrolein and caffeine were excluded because values for RAT were higher than those of other compounds with similar logP.

[b] The sample with logP = 2.00, was determined as the point at which the regression relationship changed.

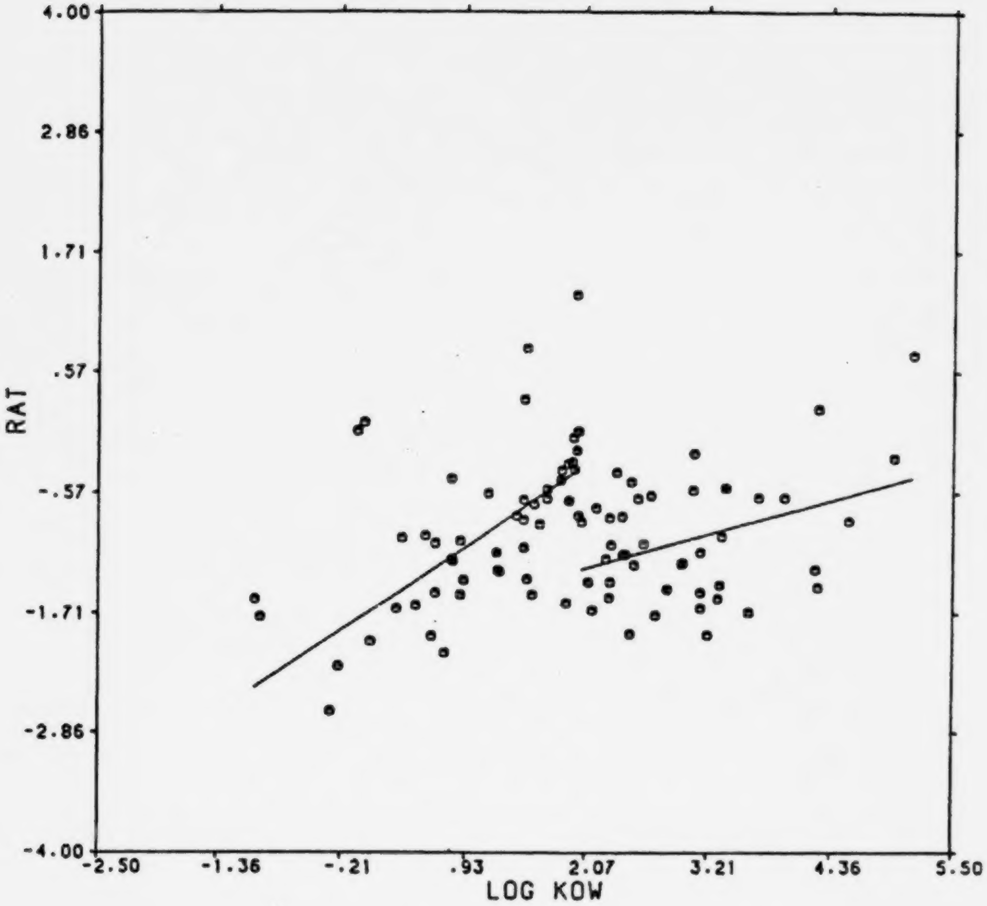

Fig. 4. Plot of RAT vs. logP (log K_{ow}) with two indicated regressions

CONCLUSIONS

This work demonstrates a high degree of collinearity between the MTOX, FHM, TEHY toxicity and octanol/water partition coefficient data over several orders of magnitude. This finding also includes some compounds known to act by specific toxic action mechanisms.

A lower collinearity is found for the algae and rat toxicity data. Although there are severe limitations imposed by the incompleteness of the data set, there is no indication to suspect substantial changes in these relationships for a more complete data set. Nevertheless, it appears most desirable to measure the most important toxicity and physico-chemical parameter values for a larger set of compounds. This set should also cover sufficient representatives of each important functional group, alone and in combination with others, and types of chemical structures.

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PREDICTION OF AQUATIC FOOD CHAIN TRANSFER OF MERCURY: DEVELOPMENT OF A NEW SIGMOIDAL MODEL

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ABSTRACT

We review some of the models proposed in the last two decades to predict the uptake of contaminants by aquatic organisms from the food chain with a special reference to mercury. Attention is focussed on the ability of these models to fit bioaccumulation curves which exhibit initial lags and subsequent sigmoidal deviations from the classical exponential curves.

A simple sigmoidal model based on the Langmuir adsorption isotherm previously developed for the adsorption of metals onto heterogenous adsorbents (Rubin and Mercer, 1981. In: Adsorption of Inorganics at Solid-liquid Interfaces, Chap. 8, Ann Arbor Science) is described in detail. This model has been applied to data sets obtained from adult shrimps (**Pandalus borealis**) feeding on Hg-contaminated food and from mussels (**Mytilus edulis**) feeding on contaminated particles. The biological significance of terms of the equation is discussed and we stress the importance of developing a deterministic model that can explain sigmoidal accumulation curves for metal uptake from food.

Note: This manuscript will be submitted to Environmental Toxicology and Chemistry by the end of August and will be close to acceptance by November. I am interested in publishing an extended abstract of this work in the Proceedings of the workshop.
My first choice is an oral presentation but I will accept a poster if platform sessions are already full.

DATA GAPS IN THE AQUATIC TOXICOLOGY OF PRIORITY PESTICIDES AND INDUSTRIAL TOXIC SUBSTANCES IN CANADA

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Abstract

Canadian Water Quality Guidelines are being developed for priority in-use pesticides and industrial toxic substances.These guidelines are based on a critical review of all currently available published and non-proprietary information on uses and production, sources to the environment, environmental concentrations, fate and persistence in various environmental media and toxicity to non-target receptors. A standardized guideline development protocol including guiding principles, minimum data requirements, derivation method and rationale has been prepared for the protection of aquatic life.

A survey of the available aquatic toxicological literature for 17 in-use pesticides and 14 industrial toxic substances revealed numerous critical data gaps which were severe enough to support only interim guidelines or prevent guideline development altogether. The most frequent data gaps encountered were the lack of chronic exposure studies for fish and invertebrates, and the lack of acute or chronic exposure studies for aquatic plants.

A broad survey of eight environmental toxicological journals from 1985 to the present was conducted in order to: (1) predict whether the data gaps specified above are likely to be filled in the near future, and (2) determine whether the recent literature includes studies of higher levels of biological organization (e.g. community) under more field-relevant conditions of exposure (e.g. chronic, multimedia) and scale of observation (e.g. enclosure, field). This latter information is essential towards reducing the uncertainty inherent in developing guidelines. The survey revealed that there were relatively few toxicological studies being conducted on aquatic decomposers, plants, non-planktonic invertebrates, and non-fish vertebrates. Further, few chronic studies were available for fish and invertebrates. Finally, studies employing a wide range of aquatic biota under field-relevant conditions were very rare in the recent literature. Clearly, the major task of the research community for the 1990s is to eliminate these information deficiencies in order that scientifically defensible environmental quality guidelines can be developed.

Résumé

Les Recommandations pour la qualité de l'eau au Canada sont en voie d'être élaborées pour les pesticides présentement en usage et jugés prioritaires et pour les substances toxiques industrielles. Ces recommandations sont rédigées d'après une critique de toute la documentation parue et non spécifique à une compagnie productrice. Ces informations portent sur les utilisations et la fabrication des produits, leur entrée dans l'environnement, les concentrations décelées, leur devenir, leur persistance et leur toxicité pour les milieux récepteurs non visés. Un protocole normalisé pour l'établissement des recommandations qui comprend les principes directeurs, les données minimales requises, la méthode de dérivation et une justification a été préparé afin de veiller à la protection de la vie aquatique.

Un examen de la documentation publiée sur la toxicologie en milieu aquatique de 17 pesticides couramment utilisés et de 14 substances toxiques a permis d'identifier de nombreuses lacunes importantes. Compte tenu de leur gravité, celles-ci ont donné lieu à l'élaboration de recommandations provisoires ou ont même empêché une telle élaboration. Les lacunes étaient surtout évidentes au niveau de la pénurie d'études traitant des effets chroniques des produits sur les poissons et les invertébrés ainsi que des effets chroniques et aigus sur les plantes aquatiques.

Un examen complet de huit revues scientifiques en matière de toxicologie du miliue parues de 1985 à ce jour a donc été effectué afin de déterminer : (1) si des recherches allaient être menées dans un proche avenir afin de réunir vraisemblablement les données manquantes précisées ci-dessus et (2) si la documentation récente traitait d'études sur des niveaux d'organisation biologique plus élevés (p. ex. : une communauté) soumis à des conditions d'exposition (p. ex. : long terme, multimédias) plus représentatives de celles présentes sur le terrain et à une échelle d'observation adéquate (p. ex. : espace circonscrit, sur le terrain). Ce dernier renseignement est essentiel pour réduire l'incertitude liée à l'élaboration des recommandations. L'examen a précisé qu'il y a relativement peu d'études toxicologiques concernant les effets des produits sur les décomposeurs, les plantes, les invertébrés non planctoniques et les vertébrés autres que les poisons. De plus, il existe peu d'études sur la toxicité à long terme pour les poissons et les invertébrés. Enfin, rares ont été les études trouvées dans la documentation récente concernant une diversité d'organismes aquatiques exposés à des conditions susceptibles d'être retrouvées sur le terrain. Évidemment, le principal défi que doivent relever les chercheurs dans les années 1990 est d'éliminer les lacunes dans l'information pour permettre d'élaborer des recommandations relatives à la qualité de l'environnement qui soient défendables du point de vue scientifique.

BACKGROUND

Environment Canada is currently developing Canadian Water Quality Guidelines for priority chemical substances (CCREM 1987, and updates). The major water uses addressed are: (a) water to support freshwater and marine aquatic life; (b) raw water for drinking water supply; (c) agricultural uses including irrigation and livestock water; (d) recreational water

quality and aesthetics; and (e) industrial water supplies. Substances are selected for guideline development according to national priorities such as the Canadian Environmental Protection Act (CEPA) Priority Substances List and Canadian Council of Ministers of the Environment (CCME, formerly CCREM) Task Force pesticide priority lists.

By providing the most current scientific information regarding the potential effects of priority chemical substances to major water uses in Canada, Canadian Water Quality Guidelines are used to:

- assist in management for the protection and enhancement of water resources by specifying safe levels for priority substances in the aquatic environment;
- assess water quality issues and concerns (i.e. "environmental yardsticks");
- establish water quality objectives at specific sites;
- provide targets for control programs;
- provide information for State of the Environment Reporting.

Canadian Water Quality Guidelines are based on a critical review of all published and non-proprietary literature currently available. In developing guidelines for the protection of aquatic life to date, a number of critical data gaps have been identified with regards to the aquatic toxicology and fate of these substances. In many cases, these data gaps were severe enough to support only interim guidelines or prevent guideline development altogether. This has led to a broader investigation of the current trends in aquatic toxicological studies in order to: (1) predict whether specific guideline data gaps are likely to be filled in the near future and (2) determine whether the recent literature includes studies of higher levels of biological organization (e.g. community) under more field-relevant conditions of exposure (chronic, pulsed etc.) and scale (e.g. enclosures, field tests). This latter information is essential towards reducing uncertainty inherent in developing Canadian Water Quality Guidelines. For the purposes of this paper, only freshwater aquatic toxicological data gaps will be discussed.

Deriving Guidelines for Aquatic Life: The Use of Safety Factors

The guiding principles, minimum data requirements, derivation method and rationale for deriving Canadian Water Quality Guidelines are detailed in CCME (1990). In general, when data requirements are met, guidelines are derived by applying an appropriate safety or application factor to the lowest observed effect level for a native aquatic species (see Figure 1).

The use of safety factors in guideline development provides allowance for several sources of uncertainty (Cairns 1986) including:

- extrapolation beyond more than one level of biological organization (i.e. single species to community);
- extrapolating from a single test species to the range of species resident in aquatic ecosystems in Canada;

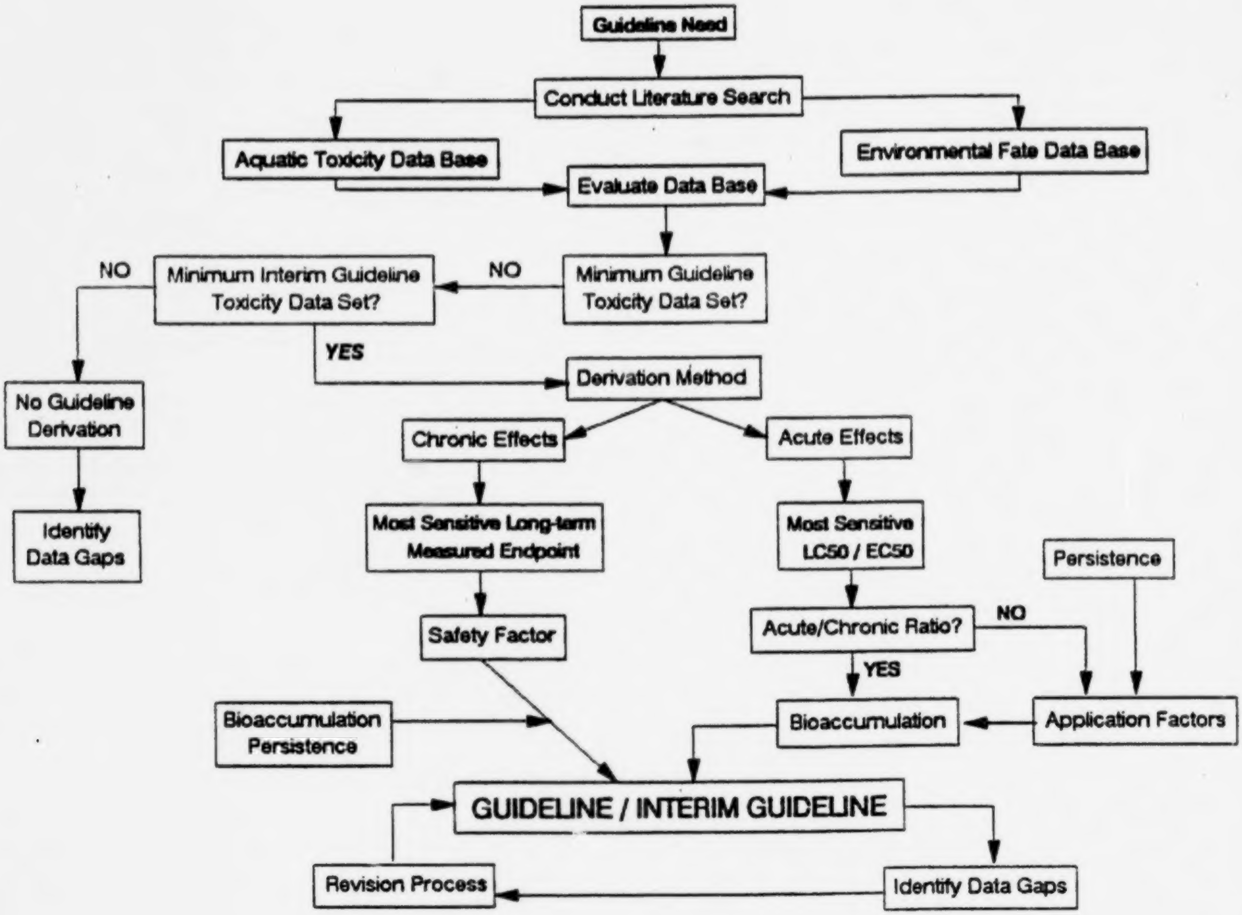
Guideline Need
Conduct Literature Search
Aquatic Toxicity Data Base
Environmental Fate Data Base
Evaluate Data Base
NO
Minimum Interim Guideline Toxicity Data Set?
NO
Minimum Guideline Toxicity Data Set?
YES
Derivation Method
No Guideline Derivation
Chronic Effects
Acute Effects
Identify Data Gaps
Most Sensitive Long-term Measured Endpoint
Most Sensitive LC50 / EC50
Persistence
Safety Factor
Acute/Chronic Ratio?
NO
YES
Bioaccumulation Persistence
Bioaccumulation
Application Factors
GUIDELINE / INTERIM GUIDELINE
Revision Process
Identify Data Gaps

Figure 1. Summary of procedure for developing Canadian Water Quality Guidelines for the protection of aquatic life.

- the lack of environmental realism in controlled laboratory tests.

To minimize the uncertainty inherent in guidelines, additional toxicity data are desirable for validation purposes. These include:

- responses from a wide range of aquatic biota;
- toxicity responses from environmentally relevant exposure conditions;
- toxicity responses from multi-media and multi-trophic level tests (e.g. microcosms, enclosures, field tests).

Minimum Data Requirements for Canadian Water Quality Guidelines for the Protection of Aquatic Life

The intended goal of freshwater aquatic guidelines is the protection and maintenance of all forms of aquatic life and all aquatic life stages. Therefore, it is essential that data from fish, invertebrates and plants be included in the guideline derivation process. For this purpose, the following minimum data set requirements have been set. Guidelines may be derived from studies involving species not required in the minimum data set (e.g. amphibians, protozoa, fungi, bacteria), provided that the minimum data set requirements outlined below are met.

FISH:

- at least three studies on three or more freshwater species resident in North America, including at least one coldwater species (e.g., rainbow trout) and one warm water species (e.g., bluegill)
- of the above studies, at least two must be chronic (partial or full lifecycle) studies

INVERTEBRATES:

- at least two chronic (partial or full lifecycle) studies on two or more invertebrate species from different classes, one of which includes a planktonic species resident in North America (e.g., daphnid)

PLANTS:

- at least one study on a freshwater vascular plant or freshwater algal species resident in North America
- for highly phytotoxic variables, the requirements increase to include four acute and/or chronic studies on non-target freshwater plant or algal species

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Evaluation of Toxicological Data

Since standard protocols for toxicity testing may become outdated or are not always available or followed, a great deal of variability exists in the quality of published toxicity data. To ensure a consistent scientific evaluation for each variable, the data included in the minimum data set should meet certain criteria.

PRIMARY DATA CRITERIA:

- currently acceptable laboratory practices of exposure and environmental controls; novel approaches will be evaluated on a case-by-case basis
- measured variable concentrations; calculated concentrations or measurements taken in stock solutions are unacceptable
- generally, static tests are unacceptable unless shown that variable concentrations did not change during the test and that adequate environmental conditions for the test species were maintained
- preferred endpoints from a partial or full lifecycle test include: effects on embryonic development, hatching or germination success, survival of juvenile stages, growth, reproduction and survival of adults
- control responses and survival must be measured and be within an acceptable range for the life stage of the test species used
- measurements of abiotic variables such as temperature, pH, dissolved oxygen, and water hardness should be reported so that any factors which may affect toxicity can be evaluated

METHODOLOGY

First, during the development of water quality guidelines for 17 priority pesticides and 14 industrial organic toxic substances, specific data gaps in the minimum data requirements were identified. Second, a wide range of peer-reviewed scientific journals[1] in environmental toxicology were selected and surveyed from 1985-present to determine the current trends in aquatic toxicology studies with respect to: classification of test organism (fish, amphibian, bird, mammal, planktonic invertebrate, non-planktonic invertebrate, algae, macrophyte, decomposer), experimental exposure medium (water, sediment, biota, multimedia), duration (acute, partial lifecycle, full lifecycle), and scale (laboratory, microcosm, enclosure, field). Each original study conducted on an industrial organic substance or pesticide was then evaluated and classified according to these latter criteria.

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[1] Archives of Environmental Contamination and Toxicology
Aquatic Toxicology
Ecotoxicology and Environmental Safety
Environmental Science and Technology
Environmental Toxicology and Chemistry
Environmental Pollution Series A
Marine Environmental Research
Marine Pollution Bulletin

RESULTS & DISCUSSION

Data gaps related to the Canadian Water Quality Guidelines minimum data requirements are presented in Table 1. The percentage of substances meeting each minimum data requirement is displayed in Figure 2. The major results were:

- Primary data on responses of plants and non-planktonic (sediment) invertebrates to industrial organic toxic substances are absent;
- Only 3 out of 14 priority herbicides included primary data from an aquatic plant study;
- Primary data from chronic studies on fish and invertebrates are lacking for many priority pesticides and industrial toxic substances.

The survey of current scientific literature over the last 5 years yielded a total of 1096 and 668 original aquatic toxicological studies for industrial organic toxic substances and pesticides, respectively. A breakdown of these studies based upon taxonomy of test species, experimental scale (i.e. laboratory, field/enclosure), exposure media (water, sediment, biota etc.) and duration (acute, chronic) are presented in Figures 3 - 9. The major results were:

- Relatively few toxicity studies are being conducted on plants and decomposers, despite their obvious importance in aquatic ecosystem food webs and energy cycles;
- The vast majority of aquatic vertebrate toxicity studies are conducted on fish species, indicating a conspicuous lack of amphibian, reptile, bird and mammal data;
- Toxicity studies on aquatic macrophytes are rare;
- Although chronic exposure studies are increasing in availability, roughly two thirds of the studies sampled were acute in duration;
- The vast majority of studies were single medium tests which were nearly always water;
- Microcosms, enclosure and field studies are rare;
- There are relatively few differences in the range of available toxicity data for pesticides and industrial organic toxic substances.

CONCLUSIONS

- Canadian Water Quality Guidelines cannot be developed for many pesticides and industrial toxic substances because of gaps in the toxicity database, particularly with regards to chronic exposure studies on fish and invertebrates and acute or chronic

Substance	Three Fish Species	One Coldwater Fish Species	One Warmwater Fish Species	Two Chronic Studies on Fish	One Chronic Study on a Planktonic Invertebrate	One Chronic Study on a Non-Planktonic Invertebrate	One Plant Species
Industrial Substances							
Trichloroethylene	●	●	●	●			
1,2-Dichloroethane	●	●	●	●	●		
1,1,1-Trichloroethane			●				
1,1,2,2-Tetrachloroethane			●		●		
Tributyltin	●	●	●		●		
Triphenyltin			●				
Chloroform	●	●	●				
Carbon Tetrachloride			●				
Dichloromethane			●				
Chloromethane							
Bromoform							
Benzo(a)pyrene		●					
Fluorene		●					
Acridine	●	●	●	●	●		
Pesticides							
Captan	●	●	●				●
Carbofuran							
Atrazine	●	●	●	●	●	●	●
Glyphosate	●	●					
Metribuzin							●
Picloram	●	●	●	●	●		●
Cyanazine							
Dinoseb	●	●	●	●			
Triallate	●	●	●		●		
Trifluralin	●	●	●		●		
Diclofop-Methyl		●	●				
Dicamba							
Bromoxynil			●				
MCPA							
Aldicarb			●		●		
Metolachlor		●	●				
Simazine			●		●		

Note: Only primary studies (substance measured, acceptable control response and methodology, etc.) were considered.

Table 1. Gaps identified in the Canadian Water Quality Guidelines minimum aquatic toxicological data requirements of 17 priority pesticides and 14 industrial organic substances. Only primary studies (substance measured, acceptable methodology and control response) were considered (circle = requirement met; blank = data gap).

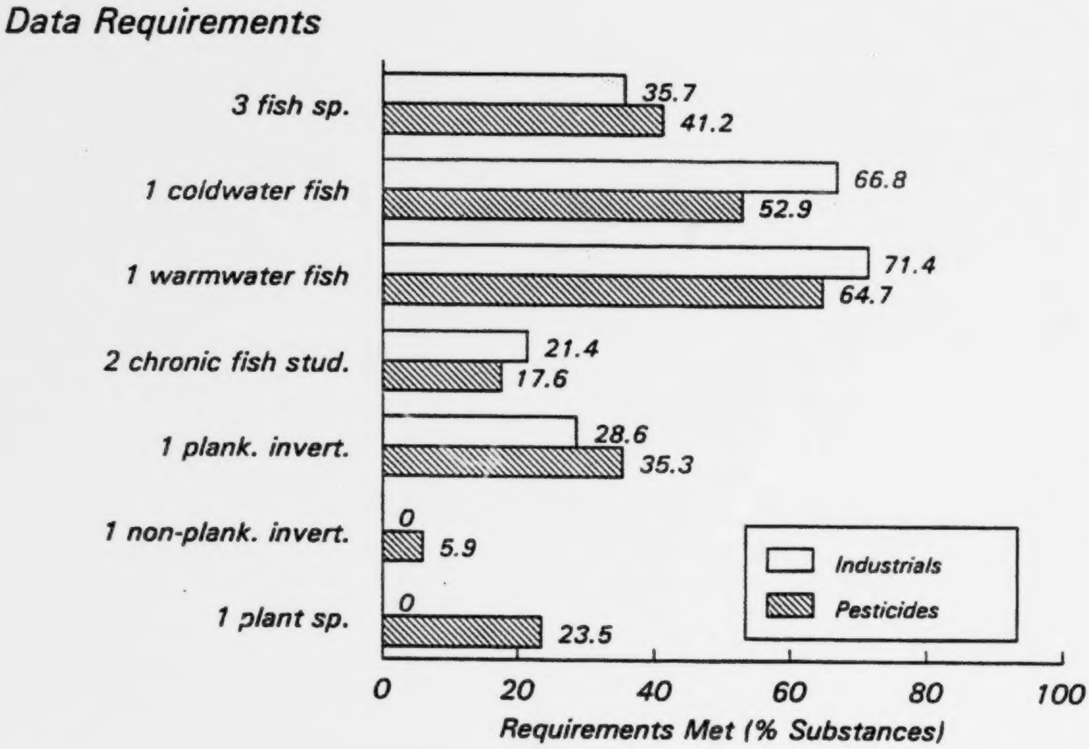

Figure 2. Percentage of Canadian Water Quality Guidelines candidate substances meeting each of seven minimum aquatic toxicological data requirements.

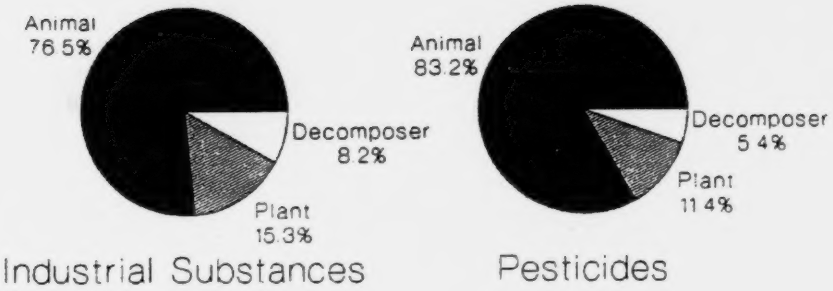

Figure 3. Taxonomic distribution (%) of test organisms used in 1764 aquatic toxicological studies on pesticides and industrial toxic substances surveyed from 1985-present.

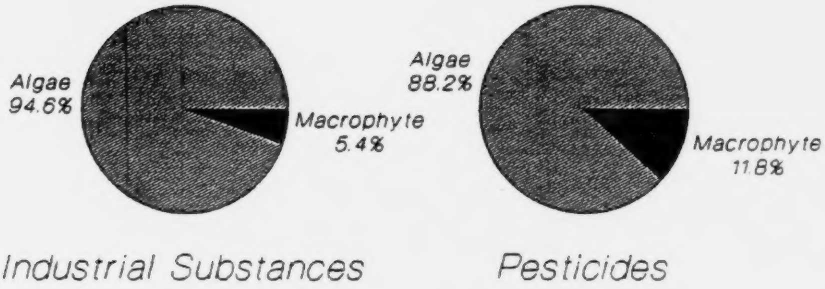

Figure 6. Distribution (%) of plant test organisms used in 244 aquatic toxicological studies on pesticides and industrial toxic substances surveyed from 1985-present.

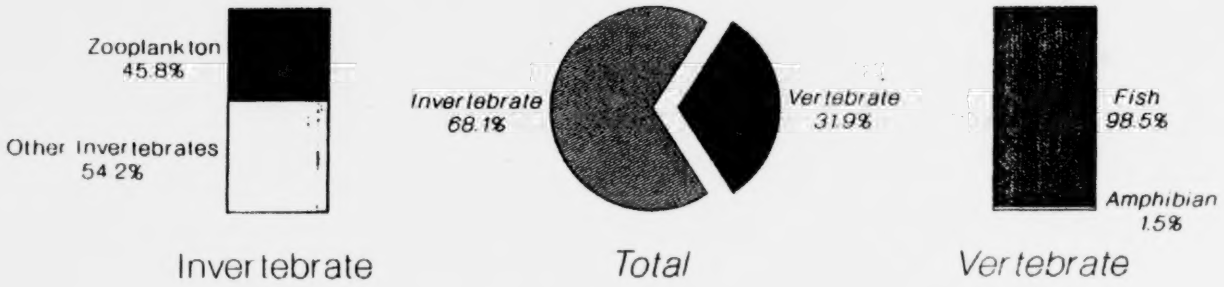

Figure 4. Distribution (%) of animal test organisms used in 838 aquatic toxicological studies on industrial toxic substances surveyed from 1985-present.

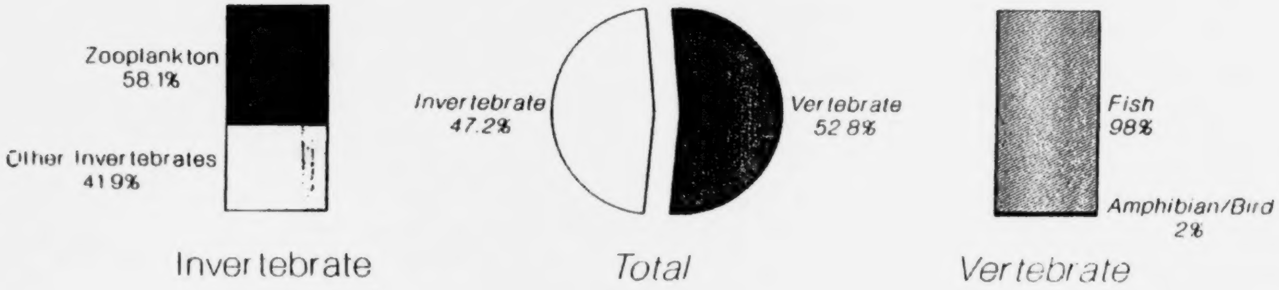

Figure 5. Distribution (%) of animal test organisms used in 556 aquatic toxicological studies on pesticides surveyed from 1985-present.

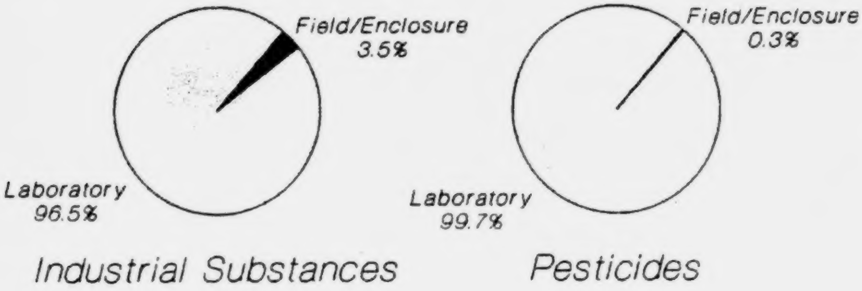

Figure 7. Distribution (%) of the experimental scale employed in 1118 and 689 aquatic toxicological studies on industrial toxic substances and pesticides, respectively, surveyed from 1985-present.

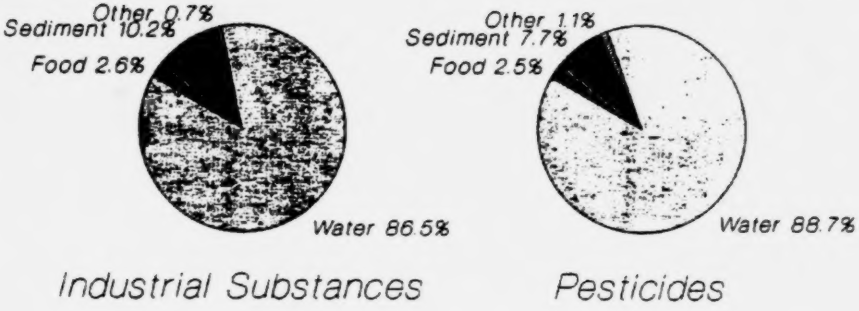

Figure 9. Distribution (%) of the experimental exposure media employed in 1116 and 689 aquatic toxicological studies on industrial toxic substances and pesticides, respectively, surveyed from 1985-present.

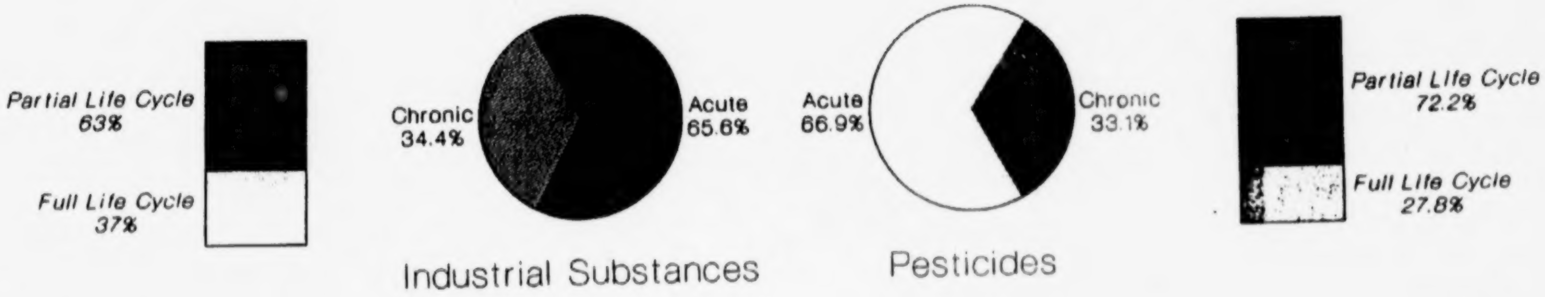

Figure 8. Distribution (%) of the experimental exposure duration employed in 1114 and 685 aquatic toxicological studies on industrial toxic substances and pesticides, respectively, surveyed from 1985-present.

studies on aquatic plants;

- A broad survey of the available aquatic toxicological data from the last five years indicates that the above data gaps are a reflection of the available scientific literature for all organic toxic substances;
- Studies employing a wide range of aquatic biota (e.g. multispecies tests) under field relevant conditions which would reduce the uncertainty inherent in guideline development are rare in the current literature.

Provision of the data to support guideline development is a major component of Environment Canada's respective Pesticide and CEPA Science Programs. Disseminating these data gaps to government research institutes, universities and the private sector is designed to provide the impetus for the provision of data needed to develop scientifically defensible environmental quality guidelines.

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COMPARING TOXICITY - QA/QC ASPECTS OF ONTARIO HYDRO'S **MISA** BIOASSAYS.

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ABSTRACT

Under Ontario's Municipal Industrial Strategy for Abatement (MISA) Program, Ontario Hydro is conducting extensive toxicity testing of effluent and process streams of nuclear, thermal and hydraulic stations from June 1990 through May 1991. Low-level radioactive effluents will be tested within Ontario Hydro but most non-radioactive effluents will be tested by private laboratories. Although the Ontario MISA program does not specify QA/QC procedures for acute toxicity tests, we incorporated a number of procedures to facilitate comparison of results.

More than 70% of the samples tested to date have been non-lethal (NL) or had an LC50 > 100%. There has been complete agreement between labs with respect to the classification of duplicate (split samples) of effluent as toxic (LC50 < 100%) and non-toxic (non-lethal or LC50 > 100%). Within labs, replicate tests have agreed well so far. LC50's were identical in more than 60% of the replicate tests with LC50 < 100%; when LC50's diverged between replicates, the variation resulted from a difference of a single mortality in one of the critical test concentrations. Both labs have experienced considerable difficulties in attempting to use zinc sulphate as a reference toxicant. We are still resolving these problems and are also looking at using 4-Chlorophenol as a reference toxicant.

INTRODUCTION

From June 1990 - May 1991, Ontario Hydro is conducting extensive chemical analysis and toxicity testing of effluent and process streams of nuclear, thermal and hydraulic stations, in accordance with Ontario's Municipal Industrial Sewage Abatement (MISA) regulations. The Biological Research Section is coordinating acute toxicity tests, which are conducted with both rainbow trout, *Onchorhynchus mykiss*, and zooplankton, *Daphnia magna.* Because of restrictions on the transport and handling of radioactive materials, low level radioactive (> 11 MBq/L tritium) samples are tested within the Section, while non-radioactive samples are tested by the external consultant (Beak). Testing started in June, 1990, and more than 225 samples had been tested by the end of September.

The Ontario MISA program states that toxicity tests must follow Ontario Ministry of Environment protocols for acute toxicity tests with rainbow trout (Craig et al. 1983) and *D. magna* (Poirier et al. 1988); however, Quality Assurance and Quality control (QA/QC) procedures are not specified. Nonetheless, we have incorporated several QA/QC procedures in our testing to facilitate comparison of the data produced between our laboratories. These include:

- standardization of laboratory procedures,
- incorporation of split samples, which are tested at both laboratories, into the sampling schedule and
- replication of the *D. magna* tests within each laboratory,
- reference toxicant testing.

LABORATORY PROCEDURES

Each laboratory maintains an up-to-date Quality Management Plan and Standard Operating Procedures. These outline the test procedures, describe the responsibilities and authorities of personnel, and document technical details for implementing specific quality assurance functions (including routine quality control procedures, audit procedures and corrective actions). Both laboratories have arranged to obtain fish, glassware and, as much as possible, other materials from common suppliers.

RESULTS

More than 70% of the samples tested to date have been non-lethal (NL) or had an LC50 > 100% (Table 1). The low number of samples which were acutely toxic (LC50 < 100%) has limited the comparisons of duplicate samples.

BETWEEN LABORATORIES - SPLIT SAMPLE TESTS

Approximately 5% of the effluent samples are collected in duplicate and tested at both labs. To date, there has been complete agreement between labs with respect to the classification of the effluent samples as toxic (LC50 < 100%) and non-toxic (non-lethal or LC50 > 100%) (Table 2). For the statistically minded, this separation is significant (χ^2 - $p < 0.01$). Although the 95% confidence limits of samples with measurable LC50's have overlapped on samples to date, the low number of samples precludes more detailed analysis.

WITHIN LABORATORY - REPLICATE *DAPHNIA* TESTS

At least 10 % of the *D. magna* tests are replicated within each lab. Replicate tests have agreed well so far, although present sample sizes are insufficient for detailed control charts. In samples with an LC50 < 100%, more than 60% of the replicate tests had identical LC50's (3 of 5 at Hydro; 4 of 7 at Beak). When the replicate LC50's diverged, the variation resulted from a difference of a single mortality in one of the critical test concentrations.

TABLE 1 - SUMMARY OF ONTARIO HYDRO TOXICITY TESTS

	TESTING LABORATORY	
	BEAK	HYDRO
Daphnia magna		
NL or LC50 > 100%	160 (78%)	37 (71%)
LC50 < 100%	46 (22%)	15 (29%)
Rainbow trout		
NL or LC50 > 100%	168 (82%)	40 (77%)
LC50 < 100%	38 (18%)	12 (23%)

TABLE 2 - SUMMARY OF DUPLICATE (SPLIT-SAMPLE) TOXICITY TESTS

Daphnia magna

		HYDRO	
		NON-TOXIC[1]	TOXIC[2]
BEAK	NON-TOXIC	8	0
	TOXIC	0	3

Rainbow trout

		HYDRO	
		NON-TOXIC	TOXIC
BEAK	NON-TOXIC	8	0
	TOXIC	0	3

---

[1] NON-TOXIC - NL or LC50 > 100%

[2] TOXIC- LC50 < 100%

REFERENCE TOXICANTS

Sodium pentachlorophenol has been used as a reference toxicant for acute toxicity tests at Beak for several years. In initial tests at Ontario Hydro laboratories, LC50's for both *D. magna* and rainbow trout were within Beak's control limits. However, because of difficulties with the use and disposal of pentachlorophenol within a highly regulated workplace, both laboratories agreed to use zinc sulphate as a reference toxicant. Both laboratories have experienced considerable difficulties in attempting to use zinc sulphate as a reference toxicant. These problems may result from the variability in zinc speciation and toxicity in water of moderate alkalinity at pH 7.5 -8.1 (Spear 1981) or nuances of experimental technique, and are still being resolved. We are also looking at using 4-Chlorophenol as a reference toxicant. We would welcome any suggestions.

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RELATIONSHIP BETWEEN SOLVENT/WATER SOLUBILITY RATIO AND OCTANOL/WATER PARTITION COEFFICIENT.

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Octanol/water partition coefficient (log Kov) is used to assess the bioaccumulative property of a chemical. Kov is usually estimated by the shake flask method although values for chemicals above 4.0 may be questionable because of the difficulty of measuring the low waterborne concentrations. Values for chemicals with Kov above 4.0 are often extrapolated from the relative retention time of a reverse-phased HPLC. This procedure does have some limitations because of the Kov values used for internal standards. An alternate method to estimate Kov was examined because many priority chemicals like PCBs have Kov values above 4.0. The solubilities for 55 chemicals were measured in octanol, fish oil, and triolein. Chemical solubility in each solvent was then related to waterborne solubility. Regression analyses indicate highly significant relationships among the log solvent/water chemical solubility ratios and Kov. It is suggested that the log solvent/ water solubility ratio of a chemical would provide a good estimate of its Kov. This method would be particularly applicable to chemicals with high Kov values because their solubilities in solvent and water should be well within the detection limits of most analytical methods.

INVESTIGATIONS OF ACUTE AND CHRONIC EFFECTS OF ALCAN SMELTER EFFLUENT ON JUVENILE CHINOOK SALMON (*Oncorynchus twshawytscha*). J. M. Beatty Spence, B. C. Environment, Environmental Protection Program, Bag 5000, Smithers, B. C. V0J 2N0 (604-847-7252).

The Alcan aluminum smelter is located in Kitimat, on the northwestern coast of B.C. The Environmental Protection Program (formerly the Waste Management Branch) has been carrying out a harbour study jointly with Alcan and Environment Canada since 1987 to determine the effects of the smelter discharge on Kitimat Harbour. The study has incorporated water and sediment analyses, beach seining juvenile fish in the vicinity of the discharge, as well as *in situ* and laboratory bioassays on juvenile chinook salmon to determine the acute and chronic toxic effects on fish. In 1988, two *in situ* bioassays were carried out. Initially a 48 hour bioassay was undertaken however, the results may have been confounded by the fact that the fish used in this study were juvenile chinook salmon which had been beach seined from adjacent areas in Kitimat Harbour. Some of the observed mortality was thought to be related to handling of the fish prior to the test.

We then decided to conduct a two week *in situ* bioassay using naive juvenile chinook salmon. Our results showed that fish held for a fixed period of time adjacent to the discharge died. In fact, all fish exposed to effluent concentrations that were found 250 metres from the outfall died within 48 hours. There were also a few mortalities of fish in the test groups 650 metres from the outfall.
Histological examination of gill tissue of the two week exposure study groups revealed that there was a deterioration in gill tissue health of the test fish that were at the stations 400 and 600 metres from the Alcan outfall. The test fish held 800 metres from the outfall and the control group showed no such deteriorations.

Polycyclic aromatic hydrocarbons (PAHs), were analyzed in composited fish muscle tissue from each of the test and control groups. Some high molecular weight PAHs were just above the detection limit in muscle samples of the group 400 metres from the outfall, but in no other groups were PAHs detectable.

The 1988 study results prompted further *in situ* work in 1989. However, a four week *in situ* study attempted in 1989, was abandoned due to vandalism in favour of a laboratory bioassay. A three week static replacement bioassay was undertaken simulating the exposure levels estimated to exist at the receiving water stations during a low tide. Our objective was to examine the chronic effects of smelter effluent on gill histology, tissue PAH levels and induction of the hepatic MFO system.

Effluent from the Alcan outfall was shipped to the B.C. Environment Toxicity Lab in North Vancouver twice a week for the replacement bioassay. At the same time, effluent was collected for chemical analysis. Juvenile chinook salmon were exposed to 0, 5, 10 and 20 percent solutions of effluent in seawater. At the end of the three week exposure period the fish were anaesthetized, and livers and gill tissue dissected and pooled for MFO analysis and histological examination respectively. The remainder of the fish carcasses were pooled, homogenized and analyzed for PAH content.

The results, although preliminary, were of considerable interest in light of the short exposure period and relatively good effluent quality during the study period. We found that there was an increasing amount of low molecular weight PAHs (specifically naphthalene and acenaphthene), with increasing exposure rate. The majority of PAHs will not be found in these tissues but rather in the bile and fatty tissue. As well, quantifying the parent PAH compound in muscle grossly underestimates the true uptake in organisms that have the ability to metabolize PAHs.

The results of gill histological examination were inconclusive, likely as a result of poor fixation technique. However, the MFO analysis was able to show that with increasing exposure concentrations, the induction of the hepatic MFO system also increased. The amount of tissue that was available for analysis was insufficient to statistically evaluate the apparent trend. In spite of this, we believe the results raise concerns regarding the pollutant levels in Kitimat Harbour and the resulting risks to the aquatic resources therein.

Based on these results, recommendations were made for upgrading effluent quality and improving the design of further studies.

Manuscript available upon request.

ACUTE TOXICITY AND ACCUMULATION OF COPPER, MANGANESE AND MOLYBDENUM BY *BASILICHTHYS AUSTRALIS*. R.G. Trucco, J. Inda, and M.L. Fernández. Facultad de Ciencias del Mar, Universidad Católica del Norte, Casilla 117, Coquimbo, Chile.

Laboratory experiences were designed under static conditions to determine the acute lethality LD50 96h of copper, manganese and molybdenum in the fresh water fish *Basilichthys australis*. Results indicate that LD50 96h for copper was 0.37 mg/L, whereas for manganese and molybdenum, [it] was over 50 mg/L. The accumulation of copper, manganese and molybdenum in muscle tissue was evaluated by bioassays at sublethal concentrations equivalent to 1% of the LD50 96h or less during long-time of exposure. Every 15 days muscle samples were analyzed and compared with control animals. Natural concentrations in wild populations that inhabit waters influenced by copper mine tailings were also determined. The element determinations in water and tissues were done by standard analytical procedure by atomic absorption spectrophotometry using a Shimadzu model AA-670 coupled with a Shimadzu graphic printer model PR-4.

GEOCHEMICAL ASSOCIATIONS OF Cd, Cu, Pb AND Zn WITH SUSPENDED PARTICULATES IN THE DON RIVER.

L. A. Warren and A. P. Zimmerman. Department of Zoology, University of Toronto, Toronto, ONT., Canada (416-978-3475).

37 suspended sediment samples were collected at 4 sites over a 12 month period on the Don River; a highly urbanized system which runs through Toronto before discharging into Lake Ontario. Geochemical associations of Cd, Cu, Pb and Zn within the suspended particulate pool were determined in 4 operationally defined geochemical phases:(1) Leachable ("exchangeable and carbonate associated"); (2) Reducible ("oxides"); (3) Oxidizable ("organics"); and (4) Residual ("mineral phases"). Metal speciation patterns were relatively constant despite high variability in absolute concentrations. With the exception of Cd, very small proportions of metal were found associated with the exchangeable fraction. Cu was the only metal found in high proportions associated with the organics fraction. The two major sediment carrier phases in the Don River are the oxides and residual minerals. As these phases form stable complexes with trace metals, the fate of trace metals in this system is intimately tied to the suspended particulate fraction. PCA analyses showed that a major proportion of the variance in metal concentrations is explained by geochemical phase.

POLYCHAETES: KEY TAXA IN MARINE ENVIRONMENTAL QUALITY MONITORING

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POLYCHAETES: KEY TAXA IN MARINE ENVIRONMENTAL QUALITY MONITORING

One primary objective of biomonitoring is to assess the impact of man-made changes on the biosphere. From a biological perspective this can be achieved in two ways: 1) by examining the body burdens and the effects toxic materials have on representative organisms, 2) by examining the biota affected by contaminants. Polychaetes serve both of these functions, in the first case, as bioassay organisms and monitors for contaminants; in the second, as pollution indicators at the species, population or community level.

This study reviews recent literature pertaining to polychaetes as species in marine environmental quality monitoring, it analyses their effectiveness in this role, and it examines the potential greater use of polychaetes as biomonitors for regulatory and marine environmental quality monitoring purposes.

Ecotoxicological Testing

The Bioassay

Polychaetes fulfil the requirements of bioassay organisms. They are indigenous, ecologically significant and easily collected. Some species are dependant upon the quality of interstitial water and sediment particles. Results obtained using polychaetes as bioassay organisms are compatible with other test species (Chapman et al. 1985, McIlroy and Means 1988, Becker et al. 1989, and Jop 1989). Polychaetes have given responses to a) heavy metals; b) organic compounds; and c) radiation; by changes in: 1) fecundity (Carr et al. 1989), 2) reproduction (Reish 1977, Jop 1989, Reish 1980, and Oshida et al. 1981); 3) growth (Røed 1981); 4) survival (Swartz et al. 1979, Pesch et al. 1986).

Indicators of Bioaccumulation

As monitoring organisms, polychaetes have been shown to accumulate deleterious materials such as a) heavy metals (Reish 1984, Bryan and Gibbs 1987), b) organic compounds such as PCB's, PAH's HCBP's (McLeese et al. 1987, and McElroy 1988) and c) organic metal complexes (e.g. organotin) (Langston et al. 1987), within their bodies, in concentrations proportional to the concentrations found in the environment. This makes them good indicators for the presence and bioaccumulation potential of these materials.

Benthic Faunal Analysis

Indicators at the Species, Population and Community Level

Polychaetes are sensitive to organic enrichment, toxic material accumulation, and heavy metal deposition, hence it follows that the presence or absence of specific polychaetes in contaminated areas are an excellent indication of marine environmental quality. Species of the family Capitellidae and Spionidae are widely accepted as pollution indicators; species of other families, e.g. Nereidae and Nephtyidae, are accepted as indicators of early successional phases in recovery Pearson and Rosenberg 1978).

At the population level, a variation from the expected distribution of year classes can indicate a stress on a population, or large numbers of one species to the exclusion of all others can indicate deleterious environmental conditions.

At the community level, numerous indices which show changes in diversity, abundance, biomass, dominance and numerical distribution have been used to show quantitatively that the community is under stress. Polychaetes are usually the most abundant organisms and account for the greatest number of species in many environments under stress and therefore are a significant element in these analyses. An Annelid Pollution Index has been devised (Bellan 1980) and used to assess pollution at a site affected by municipal sewage (Bellan et al. 1988).

An excellent use of analysis of the benthic community is to show a gradient in the community composition corresponding to a gradient in physico-chemical characteristics. This has been done from a point source for sewage (Holte and Gulliksen 1987), for hydrocarbon concentrations near a drilling rig (Addy et al. 1984) and to determine the extent of enrichment from a fish farm (Weston 1990).

Polychaetes in Multi-parameter programs

Polychaetes, both as laboratory test animals and field monitoring organisms, can be used alone or in combination with other monitoring processes such as the Benthic Triad (Long and Chapman 1985) where community structure, sediment bioassay and sediment chemistry measurements are all taken at the same site.

Recommendations for Canadian Status and Trends Monitoring Programs

Polychaetes should be:

1) used for bioassay toxicity testing
2) used as monitoring organisms for contaminants
3) used as indicator organisms at the species, population and community level, alone or as part of a multi-parameter program, once base line information on their status in Canadian waters is established.
4) The study of polychaetes should be an integral part of industrial effluent monitoring and status and trends programs to be conducted in Canadian coastal waters.

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ORGANOPHOSPHATE-HYDROLYZING ACTIVITY IN *MYTILUS EDULIS*

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INTRODUCTION

Organophosphorous acid anhydrases (OPA anhydrases) are enzymes that typically hydrolyze a variety of organophosphates including diisopropylfluorophosphate (DFP), trimethylpropylmethylphosphonofluoridate (Soman) and parathion. Organophosphates are powerful acetylcholinesterase inhibitors and are therefore used as pesticides and nerve agents.
Since Mazur discovered the first so-called DFPase in 1946, it has become apparent that OPA anhydrases are widely distributed phylogenetically. They have been found in organisms as distinct as bacteria (Chettur *et al.*,1988), mammals, cephalopods (Hoskin *et al.*,1984) and protozoa (Landis *et al.*,1985).
This report examines the operating range and kinetics of the enzymatic activity hydrolyzing DFP expressed within the digestive gland and the visceral mass of *Mytilus edulis.*

MATERIALS AND METHODS

Tissue Preparation: Mature mussels *(M. edulis)* were collected from rocks at low tide near Anacortes, Skagit County, Washington, USA and held in sea water for two hours prior to dissection on ice. The total weight of approximately 40 digestive glands and the total weight of the visceral mass from 30 individuals was determined. Tissue homogenates (33% by weight) were prepared in modified Hoskin's buffer (400 mM KCl, 50 mM NaCl and 5 mM Tris[hydroxymethyl]aminomethane, pH 7.2) using an electric blender followed by grinding with a motor-driven Elvehjem tissue homogenizer. The digestive gland and visceral mass homogenates were centrifuged (3.000 rpm, 20 min, 4°C) and 0.5ml aliquots of the supernatants stored at -15°C. In order to calculate specific activities, protein concentrations of both homogenates were determined by the Bradford method (Bradford,1976).

OPA Anhydrase Assay: A solution of 3×10^{-3} M DFP (Sigma) was prepared in 5ml of the selected buffer. Modified Hoskin's buffer was used in most assays except in the pH-range experiments. BisTrisPropane buffer (400 mM KCl, 50 mM NaCl and 5 mM 1,3-bis [tris-(Hydroxymethyl-methylamino]propane) was used at pH 4 to pH 10. DFP-hydrolysis was quantified during continuous mixing, using an Orion SA 720 ionanalyzer with a fluoride electrode. In experiments at temperatures other than room temperature, a 10 ml water-jacketed reaction vessel was used in conjunction with an immersion circulator water bath. The rate of spontaneous substrate hydrolysis was measured for several minutes before introducing 100 µl of tissue homogenate. Tetrahymena OPA anhydrase (kindly supplied by M.V. Haley, Chemical Research Development and Engineering Center, Aberdeen Proving Ground, Maryland) served as a control prior to assaying the mussel homogenates. Enzyme mediated hydrolysis was then recorded for four to ten minutes. OPA anhydrase activity was calculated, after correcting for the rate of spontaneous hydrolysis.

RESULTS

Homogenates of both the digestive gland and the visceral mass show significant levels of DFP hydrolysis, with a five-fold higher specific activity found in the former (20.84 µmoles DFP/min/g protein in digestive gland; 4.19 µmoles DFP/g protein/min in visceral mass; see Fig.1).

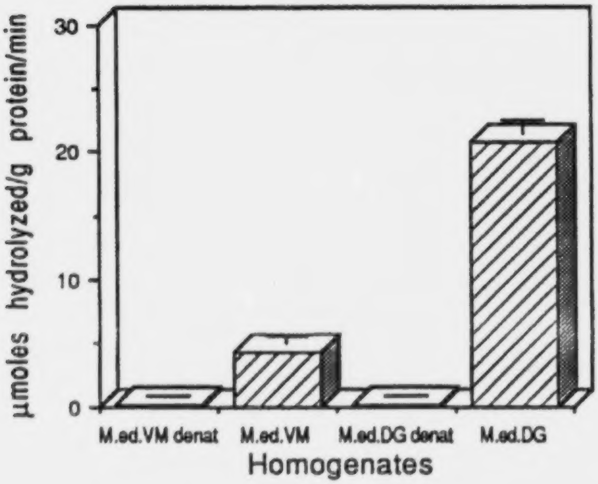

Figure 1. Hydrolysis of DFP by visceral mass and digestive gland of *M. edulis*. VM= visceral mass, DG=digestive gland, and denat= denatured by 70°C for 30 minutes.

The enzyme was thermolabile; enzymatic activity was destroyed by heating the samples for 30 minutes at 70°C. The digestive gland OPA anhydrase exhibited typical enzyme kinetics at 23°C; increasing substrate concentrations lead to an increase in reaction rate until saturation occurs These data gave a maximum velocity of 22.14 µmoles DFP/g protein/min and a K_m of 1.72×10^{-4} M. Experiments at different temperatures indicate that DFP-hydrolyzing activities increase with higher temperature for both the digestive gland and the visceral mass homogenates (Fig.2). At 55°C, homogenates show the highest reaction rate, however, the enzyme slowly loses activity after three minutes, probably due to denaturation. At 60°C, activity loss occurs within the first minute. Therefore, V_{max} and K_m should be higher when performing the assay at 30°C - 40°C, the temperature routinely used by other authors.

In preliminary pH-range experiments using two different buffers, the rate of reaction shows a tendency to increase with the pH of the buffer in the range of pH 4 to pH 9 (Fig.3).

After pH 9, the reaction rate drops remarkably. The specific ion probe could not be used at a pH higher than pH 10 due to interference from hydroxyl ions. Furthermore, the spontaneous hydrolysis rate increased drastically at and after pH 8.5, making determinations of enzyme-mediated hydrolysis difficult. In spite of these difficulties, the preliminary results suggest a pH-optimum at pH 9 for the digestive gland homogenate.

DISCUSSION

The DFP-hydrolyzing activity within *M. edulis* has demonstrated activity over a broad range of environmental conditions. It has previously been demonstrated that in the clam, *Rangia cuneata*, (Landis *et al.*,1989a, 1989b) and the protozoan, *Tetrahymena thermophila*, (Landis *et al.*,1987), OPA anhydrases exist in multiple forms. The broad range of activity with pH and/or temperature is an indication that more than one OPA anhydrase may exist within *M. edulis* as well. The different reaction rates in digestive gland and visceral mass also support this suggestion. Anderson *et al.* (1988) determined DFP-hydrolyzing activity in major tissue sites of the estuarine clam, *R. cuneata*. In *Rangia*, like in *M. edulis*, the highest reaction rate can be found in the digestive gland. This is not surprising since the digestive gland of bivalves is the

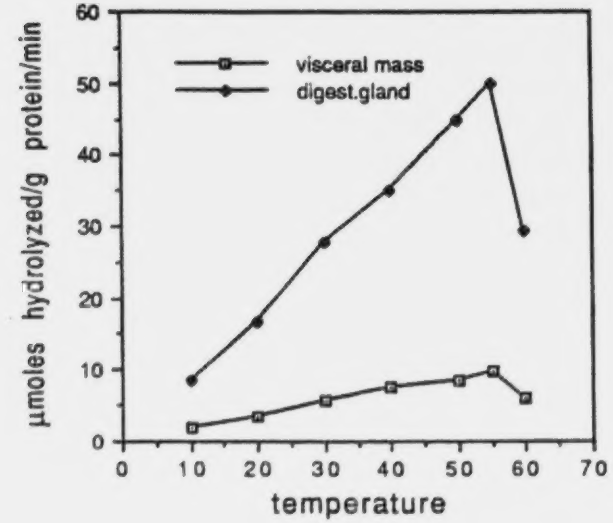

Figure 2. Activity versus temperature for the *M. edulis* - OPA anhydrase. In both tissues optima were 55°C.

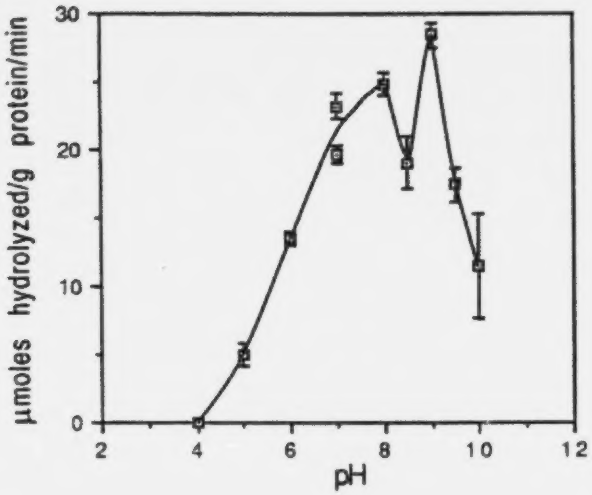

Figure 3. Activity versus pH for the *M. edulis* - OPA anhydrase. Optimum pH ranged from 8 to 9.

primary organ for bioaccummulation and biotransformation of environmental chemicals. Recently, the suggestion was made that the OPA anhydrases are involved in the metabolism of phospho- and phosphonolipids (Landis *et al.*,1985). Some OPA anhydrases may also be important in dehalogenation of naturally occurring halogenated metabolites (Landis *et al.*,1989c). Despite the uncertain physiological role of OPA anhydrases, an appreciation of their diversity is important in understanding the potential impact and risk of organophosphate xenobiotics. OPA anhydrase-research may also lead to the design of a viable system of using enzymes for hazardous waste treatment.

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EFFECTS OF DIETARY AND WATERBORNE COPPER ON TISSUE COPPER BURDENS AND WATERBORNE COPPER TOLERANCE IN THE RAINBOW TROUT (*Oncorhynchus mykiss*).

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The purpose of this study was to determine the relative contribution of dietary and waterborne Cu to both Cu tolerance and tissue burden in the rainbow trout, *Oncorhynchus mykiss*. Trout were fed either a practical reference diet (no added Cu) or a Cu-supplemented practical diet (684 mg kg^{-1}). Each diet was offered to triplicate tanks of 70 fish receiving nominal waterborne Cu concentrations of 120 $\mu g\ L^{-1}$, 60 $\mu g\ L^{-1}$, 30 $\mu g\ L^{-1}$ or 0 $\mu g\ L^{-1}$ in a 2 x 4 factorial design. During the experiment, the trout in each tank were bulk-weighed biweekly to evaluate growth performance. Liver, kidney, gill and bone tissues as well as gut contents were sampled after 42 d of the experiment. Growth, food conversion efficiency, condition and survival were not affected at any of the treatments. Dietary Cu significantly increased Cu concentrations in the liver ($p<0.001$), kidney ($p<0.001$), gill ($p=0.005$) and gut ($p<0.001$). Waterborne Cu also increased Cu concentrations in the liver ($p=0.018$). Analysis using Tukey's multiple comparison test revealed the highest water concentration had a significant impact on the liver tissue concentration compared to the lower waterborne concentrations. There were also elevated Cu levels in the kidney ($p<0.002$) of trout exposed to elevated waterborne Cu concentrations. The lowest water concentration had no effect relative to the control, while there was an increasing contribution with higher water levels. There was no effect ($p=0.930$) of waterborne Cu on the concentration of Cu in gill tissue. There was a strong effect of dietary Cu ($p<0.001$) and no effect of waterborne Cu concentration ($p=0.519$) on the Cu concentration of the gastrointestinal tract. The majority of observations of the Cu content of the bone were below the detection limit. Visual inspection of the data revealed no trends.

Trout exposed to the highest waterborne Cu concentration had elevated Zn concentrations in the liver ($p=0.025$) and decreased concentrations in the kidney ($p=0.045$). Results of the TUKEY'S multiple comparison test for kidney showed no pair-wise differences. There was no effect of dietary copper on liver and kidney Zn concentrations ($p=0.966$ and $p=0.860$).

The relative contributions of dietary versus waterborne Cu to liver tissue were analyzed. 99% of the Cu burden in the liver tissue of the fish treated with low water and high diet was from the Cu diet. As the waterborne Cu concentration increased, so too did its contribution of Cu to the liver tissue. The contribution of waterborne Cu increased in the medium water concentration to 15%. At the high waterborne Cu concentration, the contribution from the water to the liver Cu burden increased to 37%. Overall, waterborne copper exposure played a secondary role in tissue Cu burdens, although indications from liver tissue suggests that at higher waterborne concentrations, waterborne Cu would take over as the primary source of tissue Cu.

Following the six week dietary/waterborne Cu exposure period, fish were subjected to an acute lethality test. Waterborne pre-exposure significantly increased the tolerance of trout to waterborne Cu ($p=0.0001$), but only in the group pre-exposed to 127 μg Cu L^{-1}. Pre-exposure to dietary Cu appeared to decrease waterborne Cu tolerance ($p=0.0381$) in trout. No significant interaction ($p=0.2234$) effect of diet and waterborne Cu pre-exposure on lethality was evident.

NATURAL CONCENTRATION OF CADMIUM, COPPER AND IRON IN THE SCALLOP *ARGOPECTEN PURPURATUS* FROM LA HERRADURA AND TONGOY BAYS, COQUIMBO, CHILE. J. Inda and R.G. Trucco. Facultad de Ciencias del Mar, Universidad Católica del Norte, Casilla 117, Coquimbo, Chile.

This study compares the natural accumulations of cadmium, copper and iron in gonad and muscle tissue (edible parts) of the scallop *Argopecten purpuratus* from two different bays. Samples of 22 scallops, each of three different sizes: 8, 10 and 12 cm in length, were taken monthly over one year at La Herradura and Tongoy Bays. Metal from gonad and muscle tissue was extracted with (1:1) nitric acid-deionized water and concantrations were determined by atomic absorption spectrophotometry. Values are expressed in mg/Kg dry weight. Results show that cadmium concentrations were 95% higher in both tissues at Tongoy Bay with a maximum of 2.22 and 2.15 mg/Kg in the gonad and muscle respectively. Copper concentration shows higher values of 11% and 19% at La Herradura Bay, with a maximum of 35.70 and 31.50 mg/Kg in the gonad and muscle tissue, respectively. Iron concentration was 11% higher at La Herradura Bay with a maximum value of 42.35 and 41.53 mg/Kg in gonad and muscle tissue, respectively. Results are discussed in light of the anthropogenic influence in each bay.

WHEN ARE FISH STARVING? A NEED TO ESTABLISH ROUTINE INDICATORS OF NUTRITIONAL STATUS FOR TEST ORGANISMS IN FIELD CAGING STUDIES

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ABSTRACT

Hatchery juvenile coho salmon were held in situ for six weeks during baseline studies in 1988 and 1989 to assess mercury availability. Fish were fed on a fixed schedule and were able to feed on natural aquatic insects as well. Total lipid and moisture content were used to assess nutritional status. In 1988, for the in situ caged fish, a significant decrease in total lipid content was observed and moisture content increased significantly. For the hatchery stock, although a significant reduction in lipid content occurred over six weeks, apparently as a result of a 50% decrease in food ration, a corresponding increase in moisture content was not observed. In 1989, a significant reduction in lipid content occurred in the in situ caged fish. A significant increase in moisture content occurred in fish at the four in situ sites. No significant differences were observed in the hatchery stock. A concurrent loss of lipid and an increase in moisture content is symptomatic of starvation conditions and both warrant consideration as routine measurements in aquatic toxicity studies, both in the field and in the laboratory.

RÉSUMÉ

Des saumons coho juveniles élevés en pisciculture furent maintenus in situ pour 6 semaines, durant une étude de base (1988 et 1989) pour évaluer la disponibilité du mercure. Les poissons furent nourri selon un horaire fixe et avaient aussi la possibilité de se nourrir d'insectes aquatiques naturels. Le contenu total de lipides et le taux d'humidité furent utilisés pour évaluer le statut nutritionnel. En 1988, pour les poissons encagés, une diminution significative du contenu des lipides totaux fut observée de même qu'une augmentation significative du taux d'humidité. Bien qu'une réduction significative des lipides fût observée au cours des six semaines pour les stocks de pisciculture, une augmentation correspondante du taux d'humidité fût observée, apparamment le résultat d'une diminution des rations alimentaires. En 1989, une diminution significative du contenu lipidique fût observée chez les poissons encagés. Une augmentation du taux d'humidité fût observée chez les poissons aux quatre sites d'encagement. Une perte concurrente de lipides et une augmentation du taux d'humidité est symptomatique des conditions de famine et tout deux justifient l'adoption de ces mesures comme partie intégrate des études de toxicité aquatiques, sur le terrain comme au laboratoire.

INTRODUCTION

Toxicity results are often published without a description of the nutritional status of the test organisms, both before and during the test (Lanno et al. 1989). The feeding regime adopted in a toxicity study may have implications on the response and the sensitivity to a toxicant, especially during conditions of starvation (Brown and McLeay 1979; Collvin 1985; Segner 1987). Various researchers have described the effects of different feeding strategies, including starvation, on the proximate composition of test organisms (Brett et al. 1969; Denton and Yousef 1976; Weatherley and Gill 1981, 1983). Under conditions of starvation, loss of lipid reserves is accompanied by an increase in moisture content. Feeding rates for salmonids are available and provide the researcher with some guidance (Hilton and Slinger 1981). Brett et al. 1969 described a maintenance ration as that ration that just maintains the fish without any weight change.

Inniss et al. 1978 have reported on the design considerations necessary to successfully supply field in situ bioassay techniques. They reported that field bioassays generally lack the detail essential for the development of methodologies. The condition of the test organism is generally restricted to some qualitative statement "appeared to be in good condition" or observations of mortality (Inniss et al. 1978; Roch and McCarter 1984). Hence as part of a mine development baseline study using juvenile coho salmon held in situ to measure mercury availability and establish baseline hepatic metallothionein levels, the nutritional status of the fish was also monitored.

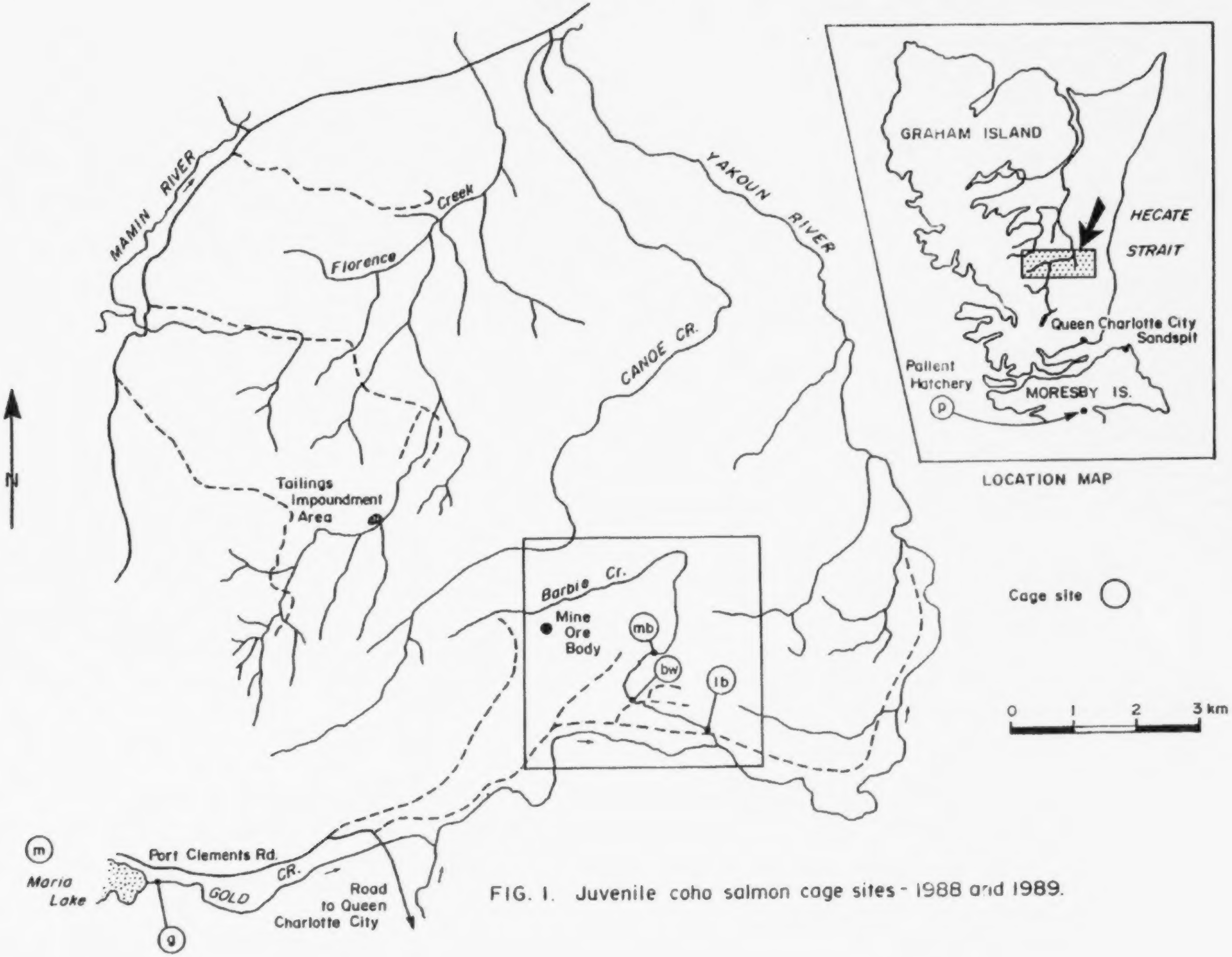
MATERIALS AND METHODS

In 1988, the juvenile coho salmon (mean weight 11.7g) used in this study were obtained from the Pallant Creek hatchery located on Moresby Island, Queen Charlotte Islands (Fig. 1). Circumstances required an alternate fish source to be used in 1989. Lake pen-reared juvenile coho salmon (mean weight 4.6g) were obtained from the Marie Lake hatchery located on Graham Island, Queen Charlotte Island.

In 1988, two study sites were located on Barbie Creek which drains the area surrounding the mine ore body (Fig. 1). An addition site control was located on Gold Creek which drains Marie Lake. In 1989, an additional site was added to Barbie Creek. The fish were transported directly to the study sites in 60L coolers with polyethylene liners. Ice packs were placed under the liners. The cages were constructed of Aqua Mesh (13mm x 13mm opening vinyl coated metal screen) and were lined with Vexar (7mm opening) to prevent any chance of escapement. Cage dimensions were 30.5cm x 30.5cm x 122cm for a volume of 110L. The cages were steam-cleaned prior to use.

In 1988, thirty fish were placed in each cage for a loading of 3.2 g/L. In 1989, forty fish were placed in each cage for a loading of 1.8 g/L. The fish were fed three times a week with a commercial fish ration (Biodiet). The feeding rate for both years was approximately 4.3% of the total cage body weight per day. The single ration was dispensed in about five minutes. The cages were suspended above the stream bottom and just below the water surface. The cages were checked for mortalities and cleaned with a plastic bristle brush after each feeding. The fish were held in situ for forty-two days.

Stream velocities were determined with a March McBirney velocity meter and never exceeded 11 cm/s. Water samples were taken at the beginning, at the mid point and at the end of the exposure period. Environment Canada, West Vancouver Laboratory methods were followed (Environment Canada 1989).


MAMIN RIVER
Creek
Florence
CANOE CR.
YAKOUN RIVER
N
Tailings Impoundment Area
Barbie Cr.
Mine Ore Body
mb
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m
Maria Lake
Port Clements Rd.
GOLD CR.
g
Road to Queen Charlotte City
GRAHAM ISLAND
HECATE STRAIT
Queen Charlotte City
Sandspit
Pallant Hatchery
p
MORESBY IS.
LOCATION MAP
Cage site
0
1
2
3 km

FIG. 1. Juvenile coho salmon cage sites - 1988 and 1989.

Dissolved oxygen was determined by Winkler titration or measured directly with an oxygen meter.

The moisture content of the muscle tissue samples for mercury analysis was determined by weighing the samples before and after freeze-drying. In 1988, skinned whole fish fillets of individual fish were analyzed but in 1989, a muscle tissue composite of four fish was used. Eight samples per site were analyzed in both years. The methanol-chloroform extraction method was used for lipid analysis (Bligh and Dyer 1959). In 1988, unskinned whole fish (less head, fins and viscera) were analyzed for lipid while in 1989, the intact whole fish was analyzed. Feeding was stopped 24h prior to the fish being removed from the cages. Five samples per site were analyzed in both years, with the exception in 1988 of site 1b d-42 where four samples were analyzed. In 1988 and 1989, feral juvenile coho salmon were also collected and treated in an identical manner.

Results are expressed as mean ± SD. Significant differences between means were detected by the Mann-Whitney test (Zar P<0.05; Zar 1984).

RESULTS AND DISCUSSION

The physical and chemical properties of the streams where the cages were located is reported in Table 1. The high total organic carbon content of Barbie Creek reflects the humic nature of the stream. The highest mean temperature was reported in Gold Creek for both years. The lowest mean dissolved oxygen levels were reported at site 1b (lower Barbie) in 1988. Stream flows were lower in 1989 and oxygen levels throughout Barbie Creek were reduced compared to 1988. Dissolved oxygen saturation levels as low as 25% were recorded in 1989.

No mortality was observed in coho salmon held in the creeks during 1988 (Table 1). Some mortality occurred at two of the three sites on Barbie Creek and at the site on Gold Creek during 1989. There did not appear to be any pattern to the mortalities observed in 1989. In both years fish appeared to be in good condition and there was no indication of fin or body abrasion. Observations of caged coho salmon stomach content indicated that the fish were feeding on the ration provided as well as on aquatic insects. Judging from the slight increase in mean body weight and fork length after 42d of caging, in 1988 and 1989, the coho salmon appeared to be on an acceptable maintenance ration (Fig. 2). However, a significant loss in the mean lipid levels of the caged coho salmon was observed after 42d exposure, in both 1988 and 1989 (Fig. 3a and 3c). The lipid results in 1988 and 1989 are not directly comparable due to differences in sample preparation. A significant reduction in mean lipid content also occurred at the Pallant Creek hatchery, apparently as a result of a 50% decrease in the daily food ration over the study period. Moisture content increased significantly in the caged coho salmon after 42d exposure, in both 1988 and 1989 (Fig. 3b and 3d). The hatchery stock moisture content did not change significantly over the 42d.

A concurrent loss of lipid and an increase in moisture content is symptomatic of starvation conditions. However, in this case the higher moisture content in the caged fish may reflect an osmotic adjustment to the water quality characteristics of the streams and reflect incomplete acclimation. The moisture content of the feral juvenile coho salmon in Barbie Creek was higher

TABLE 1: Water Quality and Coho Salmon Mortality at Cage Sites
Mean Values for Water Quality (range in parentheses)

	Sample Site (See Fig. 1)			
Variable	mb	bw	lb	g
1988				
Temperature (°C)	12.9(12.2-14.2)	-	14.8(12.9-18.3)	17.3(16.2-19.0)
Dissolved oxygen (mg/L)	8.6(8.1-9.2)	-	7.0(5.7-9.5)	9.9 (8.9-11.6)
Oxygen saturation (%)	84 (79-89)	-	71 (59-94)	106 (94-122)
pH	6.5	-	6.6	7.3
Alkalinity (mg $CaCO_3$/L)	7.1	-	7.8	13.3
Acidity (mg $CaCO_3$/L)	6.0	-	5.7	1.9
Total organic carbon (mg/L)	25	-	28	5
Specific conductance (umhos/cm)	53	-	51	44
Total Hardness (mg $CaCO_3$/L)	18.7	-	17.6	14.7
Mortality (%)	0	-	0	0
1989				
Temperature (°C)	12.8(11.2-14.2)	13.8(12.0-15.5)	14.8(12.0-17.5)	18.1(16.0-19.5)
Dissolved oxygen (mg/L)	4.1(2.9-5.0)	3.8(2.6-4.9)	3.5(3.3-3.8)	9.8(9.6-10.0)
Oxygen saturation (%)	40 (27-50)	39 (25-50)	36 (32-37)	107 (103-111)
pH	6.8	6.5	6.8	7.3
Alkalinity (mg $CaCO_3$/L)	13.0	9.7	10.2	12.9
Acidity (mg $CaCO_3$/L)	4.8	5.6	3.4	1.4
Total organic carbon (mg/L)	28	28	27	4
Specific conductance (umhos/cm)	67	60	63	50
Total Hardness (mg $CaCO_3$/L)	27.1	21.6	20.7	14.5
Mortality (%)	7.5	7.5	0	2.5

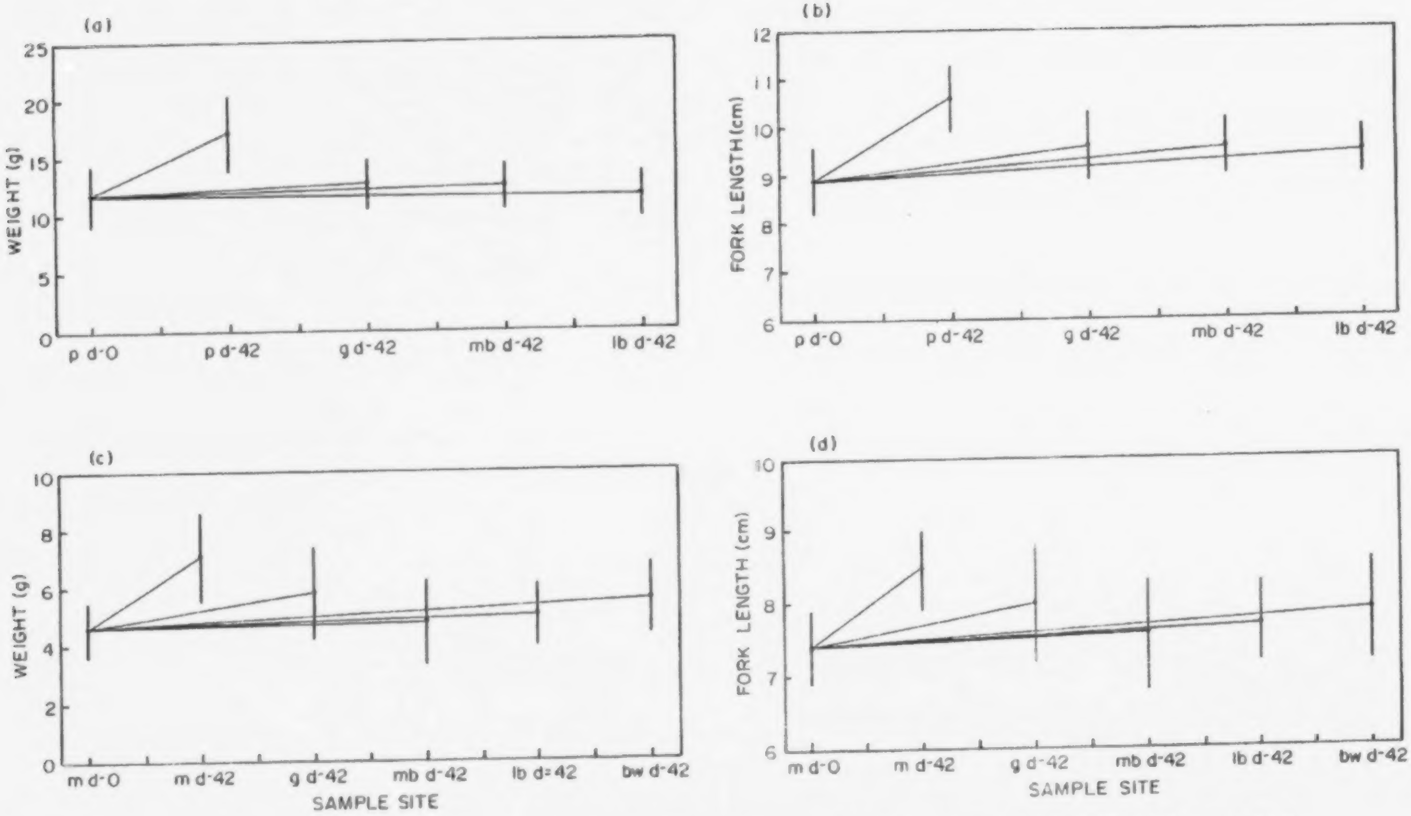

FIG. 2 Juvenile coho salmon wet weight 1988 (a), fork length 1988 (b), wet weight 1989 (c), and fork length 1989 (d). Mean ± S.D. d designates days of exposure

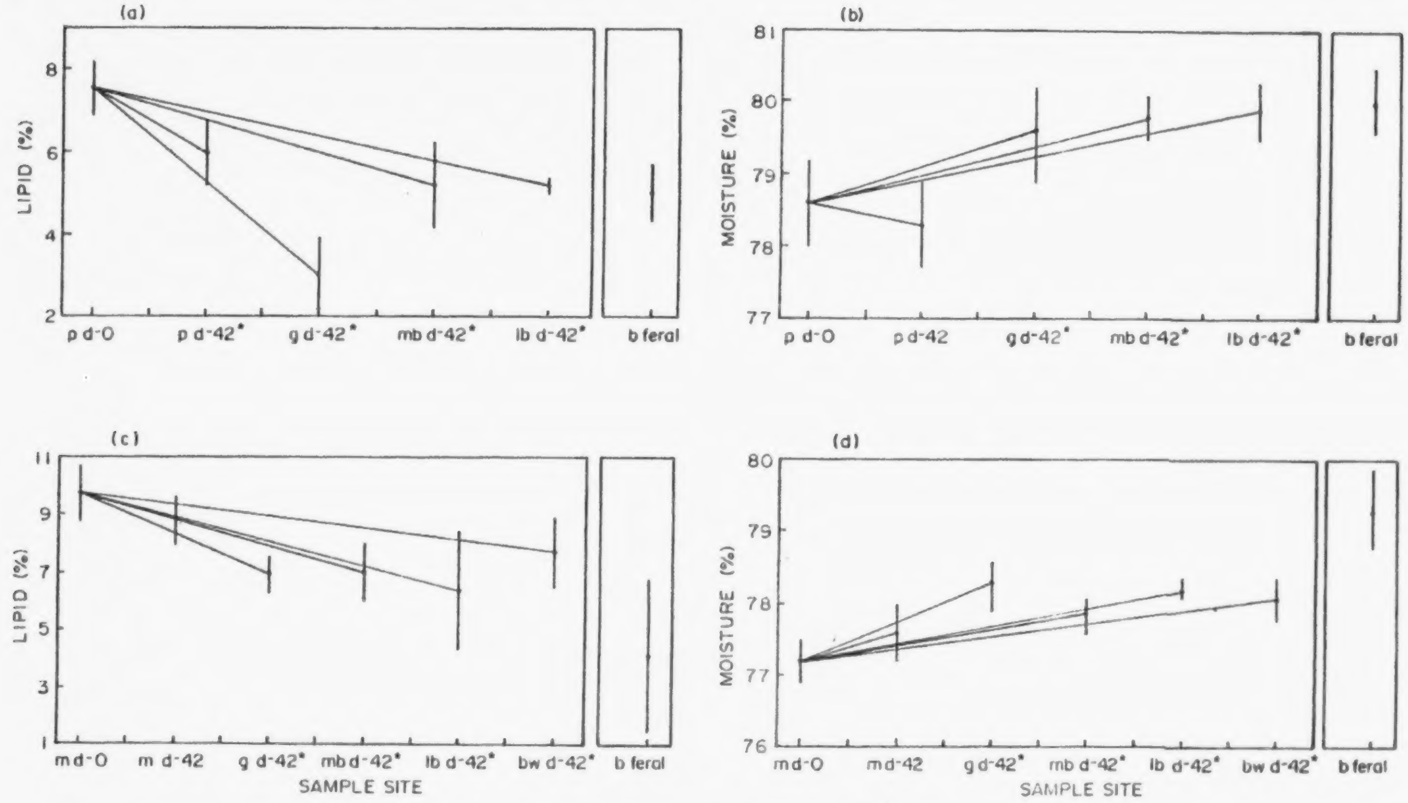

FIG. 3. Juvenile coho salmon lipid levels 1988 (a), moisture content 1988 (b), lipid levels 1989 (c), and moisture content 1989 (d). Mean ± S.D. * Significantly different compared to day-0, $p < 0.05$. d designates days of exposure.

than that of both hatchery stocks (Fig. 3b and 3d). Feral coho salmon collected from Gold Creek in 1988 had a mean moisture content of 80.2%, again much higher than the two hatchery stocks. Gold Creek drains Marie Lake where the coho salmon used in 1989 were reared. Whether the higher moisture content of the Gold Creek caged fish in 1989 is a result of starvation conditions or a reflection of an adjustment to a creek environment (as seen with Pallant hatchery stock in 1988) is not clear. The dissolved oxygen conditions in Barbie Creek in 1989 were at less than optimal levels and might be expected to impair food intake (Smart 1981). However, the level of lipid reduction was not different than for the caged fish in Gold Creek where high oxygen levels occurred (Table 1; Fig. 3c).

The significant loss of lipid in caged juvenile coho salmon in both 1988 and 1989 indicates that the fish were in a negative energy balance and that any gain in size was likely a result of this. The loss of lipid in conjunction with an increase in moisture content suggests that the fish were in a state of physiological transition, an important consideration in toxicological studies. The results of this study indicate that the routine measurement of lipid levels and moisture content in field caged fish studies gives some added insight as to the condition of the test organism. This is enhanced if the proximate condition of feral fish of the same size is also monitored.

ACKNOWLEDGEMENT

Thanks are extended to the individuals who maintained the cages during the interim periods when the author was not on site.

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AN APPROACH TO TESTING FOR ECOLOGICAL RELEVANCE USING BEHAVIOURAL TOXICOLOGY.

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EXTENDED ABSTRACT

SUMMARY

Juvenile chinook salmon (Oncorhynchus tshawytscha) were exposed to sublethal concentrations of the wood antisapstain chemical TCMTB under continuous-flow conditions. Subsequent behavioural responses of control and treated groups were examined in a 4500 L Water Column Simulator. Under vertically stratified conditions (fresh water overlying salt water) comparisons were made of distribution and swimming speed in normoxic and hypoxic waters.

Previously exposed fish displayed reduced exploratory behaviour, marked reductions of swimming speed (factor 0.25) and altered distribution under simulated estuarine conditions and in response to hypoxia. The ecological consequences of these overt behavioural changes were demonstrably linked to an increased risk of predation through experimentation.

INTRODUCTION

Changes in behaviour can be utilized as early indicators of physiological response. For example, utilizing a water column simulator (WCS) we have found that juvenile chinook salmon will begin altering their vertical distribution when dissolved oxygen drops to 70% saturation; a level well above conventional physiological thresholds (Birtwell and Kruzynski 1989).

We are currently developing techniques to apply the behavioural approach to effects of toxicants in combination with variations in salinity and oxygen; conditions which simulate estuarine waters receiving chronic waste discharges.

METHODOLOGY

Twenty juvenile chinook salmon were exposed to the lumber anti-sapstain chemical 2-(thiocyanomethylthio)benzothiazole (TCMTB) at 10 ug·L-1 for 36 h (Figures 1, 2) prior to transfer to the WCS (Figure 3) which was stratified with a layer of fresh water over sea water. Specialized lighting and a high resolution video camera provided continuous (24 h) time-lapse recordings of fish movements.

After overnight recovery, baseline behaviour was quantified. Observations included exploration activity, swimming speed, vertical location in the water column, and proportion of time spent in fresh water, halocline and sea water.

Over the next 3 h, dissolved oxygen in the fresh water layer was gradually reduced to ~3 $mg \cdot L^{-1}$. Aerated water was then re-introduced and saturation was reached within a second 3 h period. Since control and exposed fish are subjected to identical protocols, behavioural differences associated with interactions of toxicant, salinity and dissolved oxygen can be investigated.

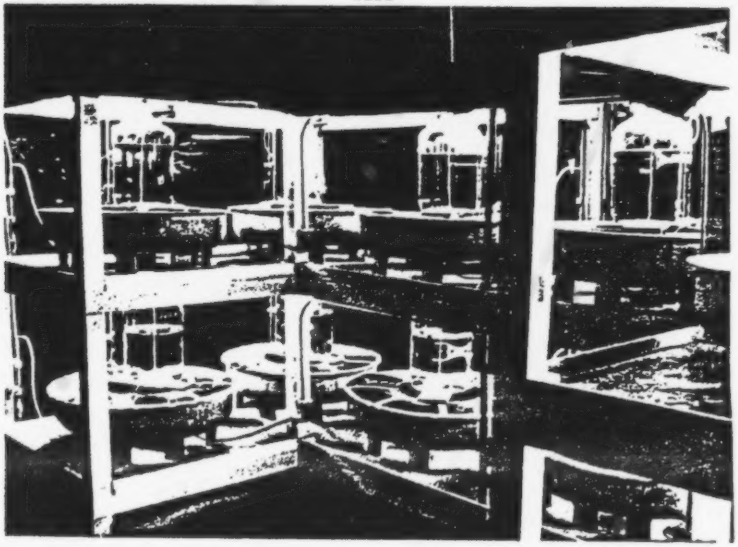

Fig. 1. Continuous flow exposure system comprising twelve 12 70 L annular exercise tanks. Each is fitted with a recirculating pump providing current velocities appropriate to size of fish being tested - usually 1-2 body lengths·sec^{-1}. Bioassays are conducted at diluent flow rates of 1-2 L·min^{-1} and can provide 90% replacement within 1.5 h. Toxicant is metered by gravity using a Mariotte bottle. Since both fresh and sea water are available, "sea water challenge" experiments can be executed without disturbing the fish. Photoperiod, adjusted biweekly, incorporates dawn and dusk periods. Very low intensity lighting provides enough illumination for fish to orient at night.

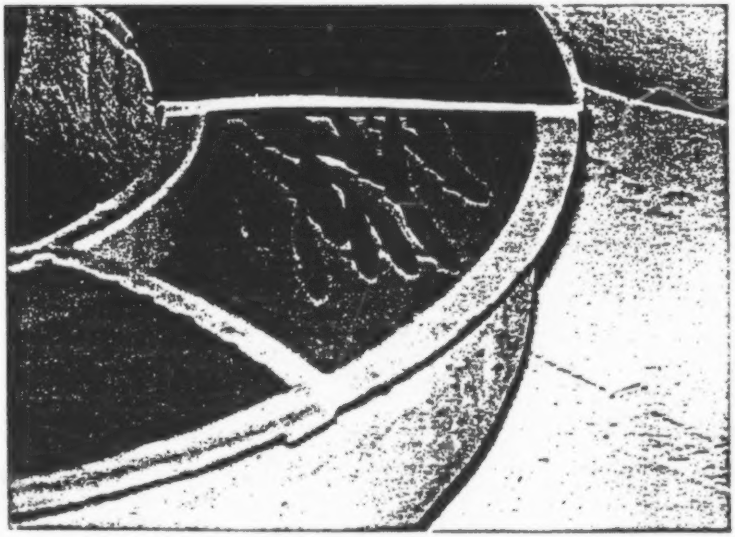

Fig. 2. Juvenile salmon shown schooling under covered area of annular tank. Discernable changes induced by toxicant exposure include schooling disruption, reduction of startle/cover response, gradual selection of areas of reduced water velocity and visual symptoms of respiratory distress. In our experience, juvenile salmonids rarely lose positive rheotaxis during sublethal toxicant exposure and although fish will eventually lose laps, they do so while pointing into the current. Initiation of lap loss can be used as a threshold for sampling, e.g., blood chemistry. Since very light MS-222 anaesthesia induces drifting, fish removal for ancillary experiments can be accomplished rapidly with minimal handling stress.

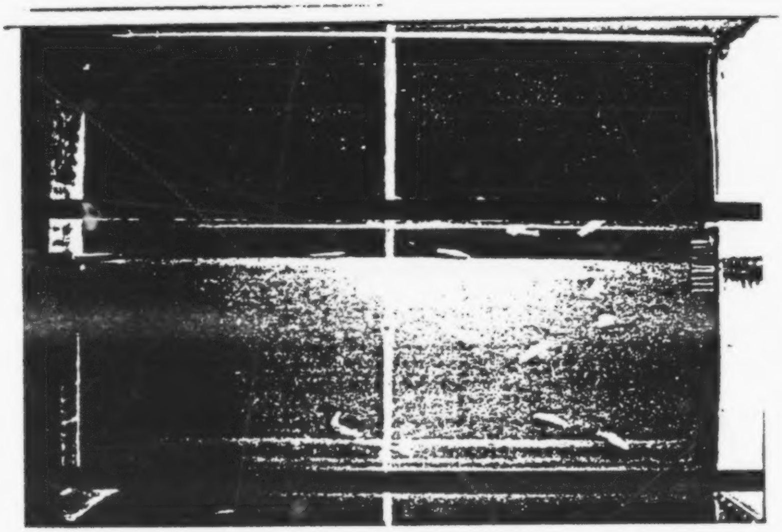

Fig. 3. The 4500 L acrylic water column simulator (WCS) showing a clear freshwater lens stratified over more turbid sea water along a sharp halocline. Daylight spectrum illumination is provided with a Light Pipe™, photoperiod control incorporates dawn/dusk periods and deep red light is used for night-time filming using a far-red (780 nm) sensitive high resolution video camera. Fish trajectories described below were plotted directly off the video screen.

In this experimental design, observations are from 10 to 16 hours after toxicant exposure and therefore represent latent behavioural effects.

RESULTS

Behavioural observations:

At termination of sublethal TCMTB exposure in annular tanks, the following observations were recorded:

- fish maintaining position against the current,
- schooling just beginning to be affected,
- fright response appears normal,
- cough rate increased, opercula flared.

After transfer to WCS:

- Exploratory behaviour and swimming speed are greatly reduced (Tables 1,2).
- Some fish move into sea water and due to inactivity, do not move back to fresh water.
- Avoidance of low oxygen still occurs but is less precise.
- Combination of stressors forces some fish against the downstream screen at low velocity (3-4 $cm \cdot sec^{-1}$). Weaker fish die (10-20%).

Table 1. Swimming speeds (body lengths$\cdot s^{-1}$) of four individual control and exposed salmon over a one minute interval (baseline) 11 $mg \cdot L^{-1}$ D.O.

CONTROL	EXPOSED
2.52	0.12
2.60	0.37
1.86	1.48
1.42	0.06

Table 2. Swimming speeds over 6-hour experiment (body lengths$\cdot sec^{-1}$).

	CONTROL	EXPOSED
MEAN	2.58	0.57
STD. DEV.	0.73	0.61
N*	104	104

* number of fish trajectories analyzed.

CONCLUSION

Latent effects of sublethal TCMTB exposure seriously disrupt normal swimming behaviour in juvenile chinook salmon suggesting that survival may be jeopardized.

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BEHAVIOURAL ASSESSMENT OF EXPOSURE OF JUVENILE CHINOOK SALMON (Oncorhynchus tshawytscha) TO SUBLETHAL DOSES OF A TOXICANT.

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EXTENDED ABSTRACT

SUMMARY

Tests based on a) the innate negative phototactic response of salmonids and b) their predator avoidance capability were used to assess the effects of a low concentration, short duration exposure (10 $ug \cdot L^{-1}$ x 36-48 h) to the toxicant TCMTB. Relative to controls, juvenile chinook salmon in the treatment condition were slower in seeking shelter from bright light, more erratic in their swimming behaviour and more likely to be eaten by predatory sculpin. These results suggest that even brief exposure to a toxicant could reduce the ability of fish to survive in the wild.

INTRODUCTION

Exposure of fish to contaminants can elicit subtle behavioural changes which may jeopardize their survival. Low (ppb) levels of the lumber anti-sapstain chemical [2-(thiocyanomethylthio)benzothiazole] - TCMTB - evoked responses in test fish which could compromise survival in the wild, for example by reducing swimming speed and exploratory behaviour. To complement these findings and to further investigate aspects of environmental relevance, we studied the movement of juvenile salmon to cover and their ability to avoid predation.

PHOTOTACTIC RESPONSES

METHODOLOGY

Two groups of 15 juvenile (~ 10 cm) chinook salmon were housed in annular tanks provided with 11°C sea water at a flow rate of 2 $L \cdot min^{-1}$. Simulated natural photoperiod, including dawn/dusk was provided. Following 48 h of acclimation, one group was exposed to 10 $ug \cdot L^{-1}$ TCMTB as Woodstat 30WB; the other was utilized as a carrier-solvent control. After 48 h of exposure, the fish were tested for escape response in the trough (Figure 1).

The trough was provided with a flow of 20 $L \cdot min^{-1}$ sea water at 11°C. Black plastic suspended over an upstream section, offered shelter from overhead broad spectrum lighting. In contrast, the downstream section, where the fish were introduced, was subjected to the full light intensity.

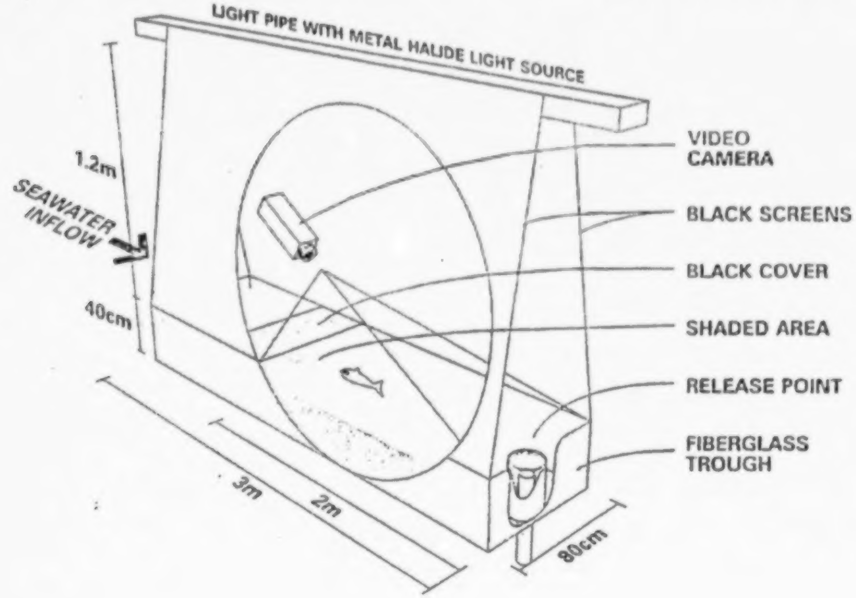
Fish were released singly from a fixed point at the drain end of the tank. The latency to escape to shelter as well as the trajectory of each escape were recorded on 8 mm format videotape. Each fish was tested only once.

RESULTS

Sample trajectories of fish in the control and the TCMTB-exposed groups are

Figure 1.

APPARATUS USED TO TEST COVER RESPONSE OF JUVENILE SALMON AFTER SUBLETHAL EXPOSURE TO THE LUMBER ANTISAPSTAIN FUNGICIDE TCMTB


LIGHT PIPE WITH METAL HALIDE LIGHT SOURCE
VIDEO CAMERA
BLACK SCREENS
BLACK COVER
SHADED AREA
RELEASE POINT
FIBERGLASS TROUGH
1.2m
SEAWATER INFLOW
40cm
3m
2m
80cm

presented in Figure 2. By inspection, the trajectories of the fish exposed to the toxicant tend to be more erratic, involving many more course changes (including direction reversals), than those of controls. Moreover, 25% of the fish in the exposed group showed no thigmotaxic response whereas all the control fish did.

Quantitative analysis of the latencies to escape showed that the exposed fish were significantly slower than their control group counterparts ($t_{one\text{-}tailed} = 2.13$, df=25, $p<0.05$). The mean escape latency of the control group was 11.0 s as compared to 32.4 s for the exposed group.

CONCLUSIONS

1. Exposure to very low (10 $ug{\cdot}L^{-1}$) concentrations of the toxicant TCMTB severely impaired the ability of juvenile chinook salmon to escape from an open space into shadows. At the same time, escape trajectories of exposed fish were characterized by more frequent changes in direction than by control subjects.

2. Exposure to sublethal doses of TCMTB can have profound effects on the innate behaviour of salmonids. Given such changes in swimming behaviour, it is possible that exposure impairs motor responses to environmental challenges through damage to the peripheral as well as the central nervous system. This raises the possibility that other deficits may exist but have remained undetected in this particular bioassay.

3. These findings also highlight the potential of behavioural techniques for the study of sublethal effects on fish. Such methods can be simple, rapid, require small sample sizes, yet provide clear insights into how environmental contaminants may affect behavioural ecology.

PREDATOR AVOIDANCE

METHODOLOGY

A mixed population comprising equal numbers of control and TCMTB-exposed juvenile salmon were transferred to the trough containing predators (Figure 3). Refuge and cover were provided by kelp *Nereocystis* sp. and PVC pipes on the bottom. Behaviour was monitored using a high sensitivity camera coupled to a time-lapse video recorder. After a standardized interval, survivors were removed, identified as to treatment and counted.

A) **Physical Conditions.** Removal of both control and exposed prey from annular tanks was facilitated with light anaesthesia (MS-222). Capture and transfer to the predation trough was done under late dusk conditions, providing just enough light for orientation and finding shelter. Darkness provided a period of overnight recovery after toxicant exposure and transfer.

B) **Selection of Predators.** Appropriate size and number were determined from preliminary experiments which defined the satiation level of the "predation unit" and a consideration of biomass (number of prey x mean weight) to be consumed within a specified time. Our current "predation unit" comprises 1 great sculpin *Myoxocephalus polyacanthocephalus*, 2 Pacific staghorn sculpins *Leptocottus armatus*, 1 Dungeness crab *Cancer magister* and 1 red rock crab *Cancer*

Figure 2. Comparisons of swimming trajectories and latencies (seconds) of individual control and TCMTB-exposed underyearling chinook salmon.

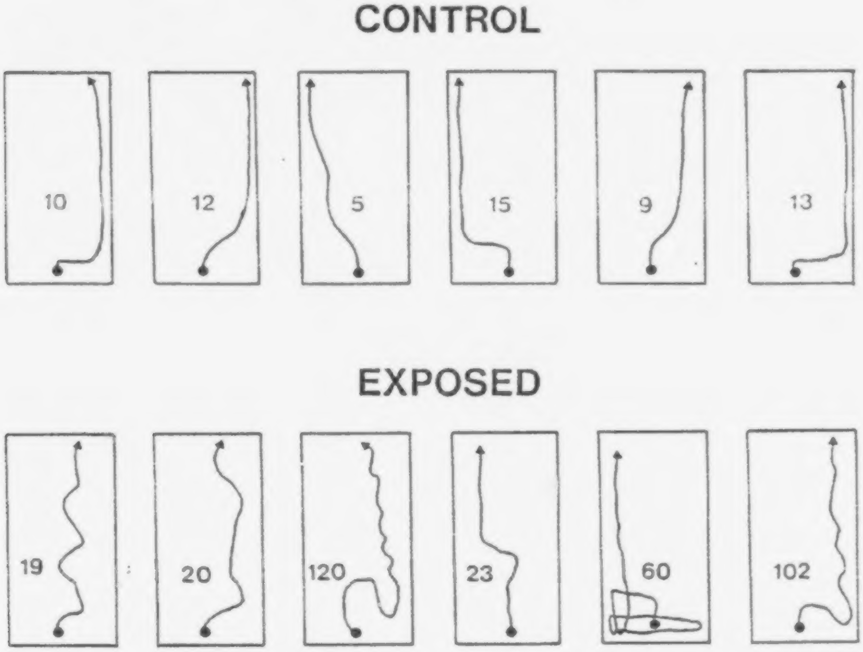
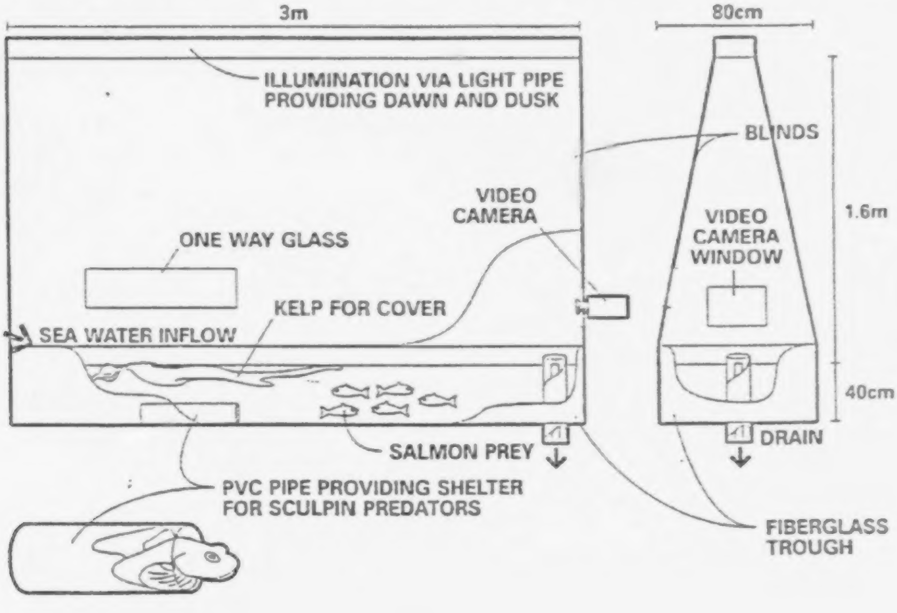

CONTROL
10
12
5
15
9
13
EXPOSED
19
20
120
23
60
102

Figure 3.

APPARATUS USED TO TEST SUSCEPTIBILITY TO PREDATION OF JUVENILE CHINOOK SALMON PREVIOUSLY EXPOSED TO TCMTB


3m
80cm
ILLUMINATION VIA LIGHT PIPE PROVIDING DAWN AND DUSK
BLINDS
VIDEO CAMERA
1.6m
VIDEO CAMERA WINDOW
ONE WAY GLASS
KELP FOR COVER
SEA WATER INFLOW
40cm
DRAIN
SALMON PREY
PVC PIPE PROVIDING SHELTER FOR SCULPIN PREDATORS
FIBERGLASS TROUGH

productus. The addition of a mid-water rockfish Sebastes sp. is planned.

C) **Selection of Prey.** Chinook salmon smolts are being studied because of their exposure to TCMTB in the Fraser River estuary; habitat critical for rearing and migration of Pacific salmon. TCMTB is leached by rainfall from treated lumber awaiting export from mills located on the banks of the river.

Pelvic or adipose fin clips are used to distinguish control (10) from exposed (10) prey.

D) **Predation "Window".** Predation begins at very early dawn of the morning following prey transfer. A period of 36 hours incorporating dawn/dusk/dawn, has proven sufficient to differentiate survival between control and exposed fish. If the duration is too long, all prey, regardless of treatment, will be consumed.

RESULTS

Table 1. Results of two trials using juvenile chinook salmon as prey.

% CONSUMPTION	OF JUVENILE	CHINOOK SALMON
TRIAL	CONTROLS	TCMTB EXPOSED*
1	40	70
2	40	90

*10 $ug \cdot L^{-1}$ x 36 h

CONCLUSIONS

Subtle behavioural changes induced by prior sublethal exposure to TCMTB can render chinook salmon more susceptible to predation.

DIOXIN AND FURAN ISOMER CONCENTRATIONS IN FISH AND SHELLFISH FROM THE VICINITY OF BLEACHED KRAFT MILLS IN CANADA

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A two year study by the Government of Canada to determine dioxin and furan emissions from bleached kraft mills included a survey by the Department of Fisheries and Oceans to measure residue levels in fish collected near the mills. Isomer specific analysis was carried out on a variety of fish and shellfish species collected in the vicinity of each of the 47 pulp mills in Canada employing a chlorine bleaching sequence. In addition, samples of fish were analyzed from the Laurentian Great Lakes where there are discharges from both bleached kraft pulp mills and from chlorophenol production. The relative concentrations of dioxin and furan isomers detected in all Great Lakes fish samples were related to the major industrial source within the specfic lake system surveyed.

A freshwater top predator fish species, walleye (Stizostedion vitreum), and sucker (Catostomus sp.), a representative bottom feeder, were analyzed on a whole fish and fillet basis to describe tissue distribution patterns of dioxin and furan. Similarly, various tissues of marine crabs (Cancer sp.) were analyzed to compare whole body levels to organ concentrations. Concentrations of all detectable dioxin and furan isomers were greater in whole fish samples compared to muscle tissue concentrations however crab organ tissue (hepatopancreas) levels were consistently greater than whole body concentrations. The proportion of whole fish to fillet and crab organ to whole body concentration ratios were similar(~7:1). This is despite the fact that levels of all measured dioxin and furan isomers were up 100 X greater in the lipid rich hepatopancreas (organ) samples compared to concentrations determined for all other tissues analyzed during the survey.

In additional related surveys, sublethal effects were measured in fish collected downstream from bleached kraft mill discharges. An elevated incidence of fin ray asymmetry in forage fish species, increased haematocrit values plus AHH and EROD induction in freshwater fish species were three specific indicators of sublethal stress measured at a number of mills discharging into freshwater systems.

In river systems receiving bleached kraft mill effluent, elevated levels of dioxin and furan isomers were detected in bottom feeding fish species collected 100 km downstream of the discharge. A significant progressive decline in contamination was observed in downstream top predator fish collections. Preliminary surveys downstream of pulp mills employing secondary effluent treatment indicate no significant reduction in enzyme induction as compared to levels measured in fish exposed to effluent with no secondary treatment.

THE PERSISTENCE AND MOVEMENT OF CONTAMINANTS FROM HEAVY OIL RECOVERY.

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The environmental behaviour of five broad groups of chemical compounds, representative of possible contaminants from the heavy oil industry, was examined using a mathematical model. The model predicts the rates of transformation, regions of accumulation, persistence times, concentrations and relative distributions in the air, water, soil, sediment and biota. It is based on the concept of fugacity, a thermodynamic term which describes the "escaping tendency" of a chemical substance.

Input parameters for the model are kinetic and equilibrium data for each compounds and a physical description of the environment including the volumes of water, soil, and sediment as well as the amount of biota, the wind speed, and river flow (if any). The fugacity approach brings some simplicity to a complex situation and permits an early assessment of the behaviour of typical contaminants in the event of an accidental release to the environment.

This paper firstly describes trends in the behaviour of some organic compounds representative of the five broad classes of chemical compounds as affected by environmental parameters. Secondly it describes two illustrative scenarios with very different hypothetical environments. The first environment is a landscape largely covered with soil and the second is mainly covered with water, representative of a lake or tailings pond.

LIQUID CHROMATOGRAPHIC AND IMMUNOLOGICAL TECHNIQUES IN ECOTOXICOLOGICAL STUDIES OF PARALYTIC SHELLFISH POISONS. A.D. Cembella, Maurice Lamontagne Institute, Dept. of Fisheries and Oceans, 850 Route de la Mer, Mont-Joli, Quebec, G5H 3Z4 (418-775-6613); G. Lamoureux, Institut Armand-Frappier, 531 Boul. des Prairies, Laval, Quebec H7N 4Z3.

Liquid chromatographic methods for the analysis of the neurotoxins involved in paralytic shellfish poisoning (PSP) are typically based upon the alkaline oxidation of these compounds to imino-purine derivatives. These oxidized derivatives can be quantified by fluorescence detection, as incorporated into either low pressure ion-exchange or high-performance liquid chromatography (HPLC) systems. An alternative immunological technique for the detection of PSP toxins, involving a competitive enzyme-linked immunoassay (ELISA) has been developed, with specificity for several of the principal PSP components, particularly saxitoxin (STX). The STX-antibody partially cross-reacts with certain common gonyautoxin analogues found in toxic dinoflagellates and PSP-contaminated shellfish. Quantitative and qualitative comparative data on the efficacity, specificity, and sensitivity of these respective methods will be presented. The potential introduction of chromatographic and immunological techniques into PSP monitoring programs, as partial replacements for conventional mammalian bioassays, represent an important advance in ecotoxicological studies on shellfish contamination and in public health protection.

IDIOPATHIC LIVER LESIONS OF ENGLISH SOLE (*Parophrys vetulus*) AND FLATHEAD SOLE (*Hippoglossoides elassodon*) INHABITING AREAS NEAR THE VICINITY OF PULP MILLS.

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Histopathological analysis of liver samples from English sole, *Parophrys vetulus* and Flathead sole, *Hippoglossoides elassodon* has revealed the presence of idiopathic lesions that are dependent on location of capture. A continuation of the studies towards the use of liver histopathology as an indicator of xenobiotics is presently being conducted, measuring the prevalence of idiopathic liver lesions in sole collected from areas contaminated with pulp mill effluents. Preliminary analysis has revealed prevalence of preneoplastic (primarily foci of cellular alterations) and neoplastic (primarily liver cell adenomas) lesions in both English and Flathead soles collected near Crofton, Port Alice, Port Mellon, Powell River and Woodfibre. Types and co-occurance of idiopathic liver lesions were not as great as that found for English sole collected from Port Moody Arm, Vancouver Harbour, an area receiving petroleum refinery effluents and having high levels of polynuclear aromatic hydrocarbons in the marine sediment. On the other hand, for the coastal reference sites, Loughborough Inlet, Rivers Inlet and Satellite Channel none of the sole collected exhibited preneoplastic or neoplastic lesions. Histopathological analysis can indicate a potential impact arising from anthropogenic chemicals derived from pulp mill and refinery effluents and is a useful measurement in determining the effects from exposure to these anthropogenic chemicals.

FRESHWATER LEECHES AS *IN SITU* MONITORS FOR CHLOROPHENOL AND DIOXIN CONTAMINATION OF LOTIC HABITAT.

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Canada (604-493-8261)

EXTENDED SUMMARY

ABSTRACT

Conventional monitoring in a stream adjacent to a chlorophenol contaminated site failed to clearly demonstrate elevated chlorophenol levels downstream of the site. Chlorophenols were below detection in many of the water, sediment and clam tissue samples.

Stream concentrations of chlorophenols were variable; water concentrations were often below detection (<0.7 PCP·L^{-1}); sediment levels were near detection limits (0.004 ug PCP·g^{-1}), as were clam tissues (<0.02-0.04 ug PCP·g^{-1} wet wt). Coarse sediments, and the paucity and distant location of clam specimens reduced suitability of these media. Low levels, and possibly intermittent release of the contaminant favoured the use of an integrative assessment method. Seven day, *in situ* bioassays using caged leeches demonstrated an order of magnitude increase in chlorophenol exposure downstream of the contaminated site. Chlorophenol concentrations at upstream and downstream locations were 0.05-0.3 ug PCP.g^{-1} and 1.20-6.1 ug PCP·g^{-1} wet weight respectively, and 0.02-0.04 ug·g^{-1} and 0.48-1.2 ug TTCP·g^{-1} wet weight respectively. Dioxin levels (octachlorodibenzo-p-dioxin) of approximately 12 ppq in the stream, corresponded to a leech accumulation level of 9.3 ppt after a seven day exposure period.

INTRODUCTION

Site History

Following the work of Hall and Jacob (1988) and Metcalfe et al. (1984), leeches were used to investigate low level chlorophenol contamination of a small stream adjacent to a wood preservation plant using pentachlorophenol for the past 22 years. Hydrogeologic evaluation of the site documented relatively flat topography with a high water table sloping towards the stream. The stream was identified as having important salmonid rearing habitat. Limited sampling of this stream had been inconclusive, with chlorophenol levels near detection limits in water and sediment.

Chlorophenol contamination of groundwater (29 mg PCP.L^{-1}) and soil (1800 mg PCP.kg^{-1}) was documented in the vicinity of the wood treatment vessels. Incomplete identification of a contaminated groundwater plume necessitated an enhanced assessment of chlorophenols in the stream. Uncertainty surrounding the continuity and timing of contaminated groundwater contact with the stream combined, with the expense of multiple media, site, and date testing of water, made leeches economically feasible integrators of chlorophenol exposure in the stream.

MATERIALS AND METHODS

In situ bioassays using leeches were conducted in 1989 and 1990 to complement limited water and sediment sampling for chlorophenols and dioxins. Leeches were collected from Black and Green Lakes; both relatively isolated, and presumed uncontaminated. Leeches were held in a glass aquarium filled with native lake water for a period of days or sometimes weeks prior to deployment to the stream. To ensure sample sizes were adequate for analysis, leeches were randomly sorted by species and size and weighed prior to placing in the enclosures. Aluminum fly screen enclosures were anchored at mid depth in the stream above and below the contaminated site. Leeches were retrieved after a period of seven days, placed in amber glass bottles on ice and frozen within 3 hours. Water and sediment samples were collected according to draft B.C. Environment protocols for pulpmill baseline sampling for dioxins and furans.

Water samples were collected once or twice during the bioassay runs to establish a possible relationship between contaminant levels in water and biota.

RESULTS

1985-1988 SAMPLING RESULTS

Sampling results for chlorophenols in water, sediment and clam tissues up to and including 1988 are shown below in Table 1.

Table 1: Chlorophenol Levels in Water, Clam Tissue and Sediment to 1988

Media		Mean	no.	Range
water[1]	u/s	<0.7	2	
	d/s	<0.8	2	
clams[2]	d/s	0.02	5	<0.02-0.04 ug/g
sediment	u/s	<0.004	2	<0.004-0.004 ug/g
	d/s	0.0075	2	0.007-0.008 ug/g

[1] pentachlorophenol only; company testing showed higher but erratic levels; lack of data quality assurance detracted from data interpretation.

[2] no detectable trichlorophenols or tetrachlorophenols were detected in clams; clams were living 8-10 kms d/s of site and no clams were found to occur upstream of the site.

1989 SAMPLING RESULTS

Results of the 1989 testing are shown in Table 2. Leech tissues downstream contained ten times greater chlorophenol levels than upstream leeches. A limited supply of leeches in this year precluded the inclusion of negative checks or replication. Despite this limitation, chlorophenol was clearly shown to be significantly higher downstream of the site than upstream.

Table 2: Chlorophenol Levels in Water, and Leech Tissue Following 7 Day *In Situ* Bioassay in August 1989.

Leech Results:	Upstream	Downstream
PCP	0.10	1.20 ug/g
T4CP	0.05	0.48 ug/g
TCP	<0.05	<0.05 ug/g

Water Results: estimates from work by Hall and Jacobs (1988) suggested PCP in water was approximately 0.6 ug/L; company testing in August-October 1989 confirmed this.

1990 SAMPLING RESULTS

Two assessments for chlorophenols and one for dioxins were conducted in 1990. The first assessment conducted in August was at two sites for chlorophenols, dioxins and furans. The second assessment was conducted in September for chlorophenols at three sites, one upstream, one mid-site, and one downstream of the contaminated site. The pH of the stream remained at 7.7 during the two studies, but was 19°C in August and 14°C in September. Water analysis and *in situ* bioassay data for 1990 are shown below in Table 3.

Table 3: Chlorophenol Levels in Water and Leech Bioassay Results for 1990

AUGUST 1990

Leech Bioassay:	Upstream	Downstream
PCP	0.05	1.8 ug/g (ppm)
T4CP	0.02	0.52 ug/g
TCP	<0.01	0.01 ug/g
Octachlorodibenzo-p-dioxin	n/a	9.3 pg/g (ppt)
Octachlorodibenzofuran	n/a	<3.2 pg/g

negative check - 0.02 ug/g PCP; <0.01 ug/g T4CP and TCP

Water Analysis:	Upstream	Downstream
PCP	<0.1	1.2 ug/L (ppb)
T4CP	<0.1	0.3 ug/L
TCP	<0.1	<0.1 ug/L
Octachlorodibenzo-p-dioxin	< 23	12 pg/L (ppq)
Octachlorodibenzofuran	< 18	10 pg/L

SEPTEMBER 1990

Leech Bioassay:	Upstream	Mid-site	Downstream
PCP	0.30	6.1	6.0 ug/g
T4CP	0.04	1.4	1.2 ug/g
TCP	0.03	<0.01	<0.01 ug/L

negative check - <0.01 ug/g PCP ; 0.03 ug/g T4CP ; <0.01 TCP

Water Analysis:	Upstream	Mid-site	Downstream
PCP	<0.1	4.4	3.3 ug/L
T4CP	0.1	1.2	1.0 ug/L
TCP	<0.1	<0.1	<0.1 ug/L

CONTROLLED BIOASSAYS

Seven day bioassays were performed at the Biotoxicity Lab in North Vancouver, using stream water from the study site, spiked to approximately 0, 0.1, and 1.0 ppb PCP. Water samples were maintained at 14°C and pH 7.5. Water volumes used for the bioassays were analyzed for PCP following the exposure period. Results of these bioassays are shown in Table 4 below. It was hoped that these simple tests would provide reference points of PCP accumulation that could be used to the field results. As the results show chlorophenol uptake was lower in the lab than *in situ.* Loss of solution strength over the 7 day period may have accounted for part of the reduce bioconcentration in lab bioassays.

Table 4: Controlled Seven Day Leech Bioassays Using Spiked Solutions of 0.7 and 0.06 ppb PCP.

Water Spiked to 0.7 ug/L

	Before	Following	Leech
PCP	0.7 ug/L	0.2 ug/L	0.20 ug/g
T4CP		<0.1 ug/L	0.02 ug/g
TCP		<0.1 ug/L	<0.01 ug/g

Water Spiked to 0.06 ug/L

	Before	Following	Leech
PCP	0.06 ug/L	<0.1 ug/L	0.06 ug/g
T4CP		<0.1 ug/L	0.02 ug/g
TCP		<0.1 ug/L	<0.01 ug/g

Negative Check Bioassay - Water <0.01 ug/L PCP

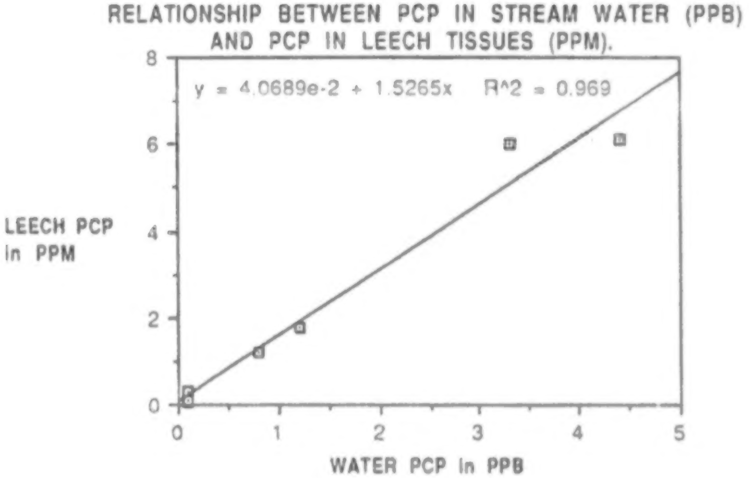
Levels of chlorophenols in Leech Tissues:

PCP	0.07 ug/L
T4CP	0.03 ug/L
TCP	<0.01 ug/L

RELATIONSHIP BETWEEN PCP IN WATER AND LEECH TISSUES

Figure 1 shows the relationship between PCP in water and PCP levels in leech tissues after a 7 day bioassay. The figure includes all data for 1989, and1990 *in situ* bioassays.

FIGURE I.



DISCUSSION

Use of leeches as *in situ* monitors for chlorophenols, served a number of purposes in the environmental impact assessment at this contaminated site. These are as follow:

1. Leeches were found to be a good initial screening technique, revealing more clearly the differences in chlorophenol exposure downstream of the site. Use of leeches in this instance overcame the problems of detection limit restrictions on water samples and the cost of multi sample programs required to detect potentially sporadic contaminant exposure in the aquatic environment.

2. Bioconcentration of PCP by leeches demonstrated a predictable relationship to PCP levels in water; levels of PCP in leeches were approximately 1000 times ambient concentrations. This information will aid assessment of the site clean up success.

3. The limited dioxin information suggests that leeches may also bioconcentrate dioxins and could be considered as possible *in situ* monitors for dioxins. This would be particularly valuable, given the expense of dioxin testing, and the need to provide an integrative measure of dioxin exposure at sites where intermittent release, of these contaminants, occurs to the environment.

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ASSESSMENT OF HABITAT QUALITY WITH BIOCHEMICAL AND HISTOLOGICAL INDICATORS IN THE ST. LAWRENCE RIVER.

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Extended Summary

Sub-lethal indicators were used to assess the environmental quality of three major riverine lakes (St-François, St-Louis, and St-Pierre) and in the middle estuary (Québec City to Saguenay River) of the St. Lawrence River from August to October 1989. The objectives of the study were to evaluate the present quality of the aquatic ecosystems of the St. Lawrence River using biochemical and histological indicators and to compare their responses to environmental quality indices (sediment and tissue contamination). In addition, the relevance of these responses for a future St. Lawrence River monitoring program must be examined. Fish species studied were White Sucker (Catastomus commersoni), Northern Pike (Esox lucius), Brown Bullhead (Ictalurus nebulosus), and Yellow Perch (Perca flavescens) in the lakes, and Rainbow Smelt (Osmerus mordax), and Atlantic Tomcod (Microgadus tomcod) in the estuary. Activity levels of a hepatic Mixed Function Oxydase (**MFO**), Aryl Hydrocarbon Hydroxylase, analyzed by spectrofluorometry (Luxon 1987), were used to assess the organisms'response to organics (Payne 1987). Concentrations of the protein metallothionein (**MT**), determined by a silver-saturation method (Gagné 1990), were used as indicators of exposure to certain metals (Klaverkamp 1984). Semi-quantitative histological observations were performed in gill and liver tissues. Contaminant concentrations in tissue were metals, organochlorine pesticides, polychlorinated biphenyles (PCB) and polynuclear aromatic hydrocarbons (PAH). Sediment contamination indices were obtained by kriging (Clark 1979) means of ratios computed as [contaminant]/[guidelines] of the Advisory Committee on Sea disposal, for all sites where sediment contamination data were available in the literature.

Highest **MFO** activities were measured in Lake St-Louis (Beauharnois region) for White Sucker and Northern Pike, and in Charlevoix (middle estuary) for Rainbow Smelt. Highest concentrations of **MT** were determined in Lake St-François (Cornwall region) for White sucker, in Lake St-Pierre for Northern Pike and Brown Bullhead, and in the south shore of the middle estuary for Rainbow Smelt. Histopathological investigations of gill and liver showed that tissue alterations were present in all species but were more pronounced in St-François and St-Louis lakes for White Sucker and Northern Pike. Also, the gills and liver of Atlantic Tomcod from the middle estuary showed evident signs of alteration. Tissue contamination of White Sucker and Northern Pike by PCB was important (2.0 mg/kg) in St-Louis and St-François lakes. High levels of mercury were measured in flesh of northern pike from the south shore of Lake St-Louis. Concentrations of chromium, copper and nickel were important in Rainbow Smelt (homogenate of 10 fishes) from the middle estuary (near Quebec city). Arsenic concentrations were high in Atlantic Tomcod (homogenate of 10 fishes). Sediment contamination indices, computed from data

available in the literature, were high in Lake St-Louis (Beauharnois region) for both organics and metals. No significant correlation was found among MFO, MT and sediment contamination indices.

The biochemical and histological indicators used in this study provide relevant information on the general quality of their environment. However, for an efficient habitat monitoring, other bioindicators (e.g. invertebrate, algae) should be considered, in addition to fish, due to the high variability of responses and the specificity of indicators to certain chemicals or stress agents. Also, the integration of a control site (non-contaminated) is necessary to assess the extent of environmental degradation.

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USE OF THE BUDS OF THE ANEMONE *AIPTASIA PALLIDA* IN MARINE TOXICITY TESTS.

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A new bioassay has been developed at the Bermuda Biological Station for Research using buds from a clone of *Aiptasia pallida*, which can be readily cultured in the laboratory. *A. pallida* produces genetically identical individuals by pedal laceration. Video analysis revealed a precise sequence of bud development through five clearly defined stages in 9 days at 25°C. This development sequence was used as a bioassay for the toxicity of copper. The bioassay was sensitive to $[Cu^{2+}]$ at 10 g/l, and is being applied to studies of other metals and to the toxicity of incinerator ash leachate.

WATERBORNE RESIN ACID UPTAKE BY RAINBOW TROUT. A.J. Niimi, Department of Fisheries and Oceans, Burlington, ON, Canada (416) 336-4868; and H.B. Lee, Environment Canada, Burlington, ON, Canada.

Subadult rainbow trout were exposed to nine resin acids to estimate their bioconcentration factor (BCF). These included abietic (Ab), neoabietic (Neo), dehydroabietic (DeAb), palustric (Pal), chlorodehydroabietic (Cl), dichlorodehydroabietic (Cl_2), pimaric (P), sandaracopimaric (SaP) and isopimaric (IP) acids. Fish were sampled after 5, 10, 14 and 20 day exposures to 1-4 μg/L of each resin acid. Pal was not detected (<.20 μg/kg) in any of the whole fish samples. The highest BCFs were observed at the 10-day sample interval after which values declined between 20-60%. This decline could suggest a biochemical response to enhance its elimination. BCF values, estimated primarily from the 14 and 20 day sample intervals, ranged from 20-30 for IP and CL; 50-100 for SaP, P, Ab, DeAb, and CL_2; and 160 for Neo. Fish were also sampled 5, 8 and 11 days after resin acid exposure ceased. No resin acids were present in these fish. The relatively low BCF values and rapid elimination rate would suggest free resin acids would not accumulate through the food chain, and waterborne uptake would be the primary mode for accumulation by fish.

EXTENDED SUMMARY

THE OCCURRENCE OF LIVER TUMORS IN NORTH SEA FLAT FISH AND A COMPARISON WITH NORTH AMERICAN STUDIES

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An epidemiological study of liver abnormalities in dab (*Limanda limanda*) and flounder (*Platichthys flesus*) was carried out in Dutch coastal waters between 1985 and 1989. An attempt was made to relate disease prevalence (in the number of individuals showing signs of the disease at a moment in time) to environmental pollution.

Both the fish species studied are flatfish of the family Pleuronectidae, but they usually occupy different habitats.

Dab are widely distributed throughout the North Sea in both coastal and offshore habitats, and are fairly sedentary, their feeding and spawning grounds overlapping. Flounder spawn in offshore areas but grow up and feed in estuaries and fresh water: after spawning in winter they usually return to the same feeding areas as the previous year, and move little during the summer. The tendency to remain faithful to the same feeding areas, together with their abundance and wide distribution, and susceptibility to disease, has resulted in both species being recommended for studies of fish disease in relation to pollution.

Over 5 years, a total of 7833 dab (minimum length 15 cm) were examined for liver abnormalities at 5 sites during late winter, and a total of 9938 flounder (minimum length 20 cm) were examined during late summer at 9 coastal, estuarine and fresh water sites. During the initial examination of grossly visible features, the presence of distinct nodules having a diameter of at least 2 mm was recorded. Histological examination of all affected fish confirmed that these liver nodules corresponded to pre-neoplastic of neoplastic lesions, including hepatocellular adenoma and carcinoma.

The prevalence of liver nodules in both dab and flounder increased with length and, at the same time, prevalence was higher in females of the affected length groups than in males. Nodules were found mainly in individuals older than 3 years of age. However, an exception to this general trend occurred recently at one polluted site, where the prevalence of nodules in young flounder (age 1) was considerably higher than in older individuals. This suggests both extreme exposure to causal agents and early mortality from the disease.

The spatial pattern of liver nodules in dab appears to show an association with pollution, in that the 2 sites with the highest prevalences are also the most polluted, one by the plume of the river Rhine and the other by the disposal of titanium dioxide wastes. No apparent relationship with the PAH content of sediments in the area was found.
However, a detailed statistical analysis (taking into account the effects of sex and length) failed to reveal significant spatial variation, partly perhaps because of the scarcity of large individuals at some sites on some occasions.

In contrast, the prevalence of nodules in flounder shows considerable spatial variation, and the differences among sampling sites were very consistent from year to year. Highest prevalence values were found along the North Sea coast near the mouths of heavily polluted estuaries with significantly lower values in a less polluted marine site; particularly remarkable is the virtual absence of the disease from brackish and fresh water irrespective of pollution status. The scarcity of large old fish in these areas only partly accounts for this absence.
At the coastal sites, the prevalence of nodules in flounder was correlated with the concentration of PAH in the sediments.

It appears likely that liver nodules in both dab and flounder have a complex multifactorial aetiology, with chemical pollution acting as one of many causal factors. A number of relevant findings still require explanation.

Firstly, the disease is absent from flounder inhabiting fresh or brackish waters, suggesting that a factor associated with salt water is necessary for its development. Secondly, similar temporal trends in prevalence in both dab and flounder over a relatively wide area, with a peak in 1987, suggest that natural factors may contribute to the occurrence of the disease. Thirdly, liver nodules are significantly associated with other diseases (epidermal hyperplasia/papilloma and lymphocystis) in dab; their aetiology must therefore have elements in common, even though lymphocystis, at least, is infectious. Finally, a possible infectious aetiology of liver nodules is supported by the observation that flounder with nodules occur in local "clusters".

Histologically, the liver lesions observed in dab and flounder correspond to those described from North American flatfish species. However, in contrast to American findings, nodules appear to be present in fish from less polluted sites as well as from heavily polluted areas in the North Sea, although it should be pointed out that background prevalences of the disease in dab and flounder from genuinely "clean" sites are unknown.

American studies have assembled an impressive body of evidence to show a direct relationship between PAHs and liver abnormalities. In contrast, epidemiological studies seem to show that liver diseases in dab and flounder in Dutch coastal waters have a more complex aetiology, with chemical pollution being only one of many causal factors.

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BIOAVAILABILITY OF METALS, VERSUS ABIOTIC AND BIOTIC FACTORS, IN HUMIC LAKES SURROUNDING THE RÖNNSKÄR SMELTERS (N.SWEDEN).

By

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BACKGROUND

The Rönnskär smelters since 1930 account for large and diverse outlets of metals to the atmosphere. They give rise to far reaching gradients of metals in surrounding land, and water bodies (Fig.1). The multitude of lakes together with large and mutually independent variations in pH(5-7) and water metal content(10:1), makes the Rönnskär area well suited for the study of dose response relationhips. The water quality is soft (15-25 mg CaCO3/l) and mesohumic to polyhumic (50-200 mg Pt/l; 7-20 mg TOC/l).

The interpretation of data is facilitated by high representativity in space and time casued by synoptic sampling, small temporal variations in water metal content, and high degree of stationarity in biota. The exposure level was measured as total water metal content, and (sometimes) filterable and dialysable metals.

Biota were sampled during late summer, when high water temperature facilitated uptake of metals. Used accumulator organisms were among others: Aquatic moss, Fontinalis antipyretica; Water Hog-

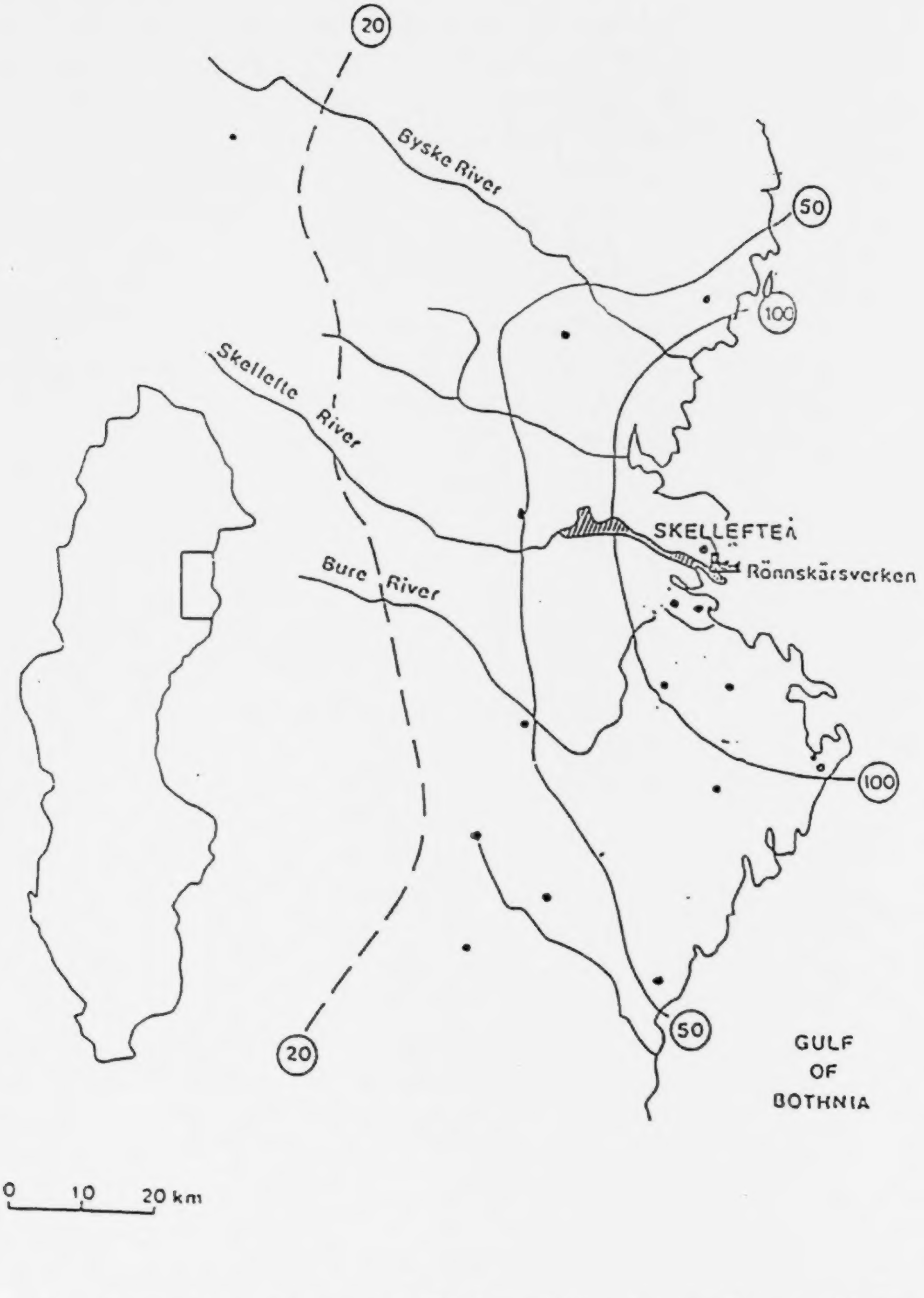
20
Byske River
50
100
Skellefte River
SKELLEFTEÅ
Rönnskärsverken
Bure River
100
50
20
GULF
OF
BOTHNIA
0
10
20 km

Fig. 1 Sampling sites and Cu-isopleths in surface sediment (0-1 cm)

louse, Asellus aquaticus; Euroasian Perch, Perca fluviatilis; and Northern Pike, Esox lucius. The samples were composed of moss tips, whole Asellus (with emptied guts), and female Perch liver or muscle (As).

RESULTS AND DISCUSSION

The results are schematically presented i figure 2. This diagram shows the relative strength of correlation for individual metals, when concentrations in biota are plotted versus ambient levels and pH (source; se references).

As can be seen from figure 2, the covariation between metal content in biota water metal content was most pronounced for Cu, Cd and As and pH >6. These relationships were most uniform in fish liver or muscle(As).

Copper revealed a bioaccumulation "threshold" in perch liver, at 2-4 ug/l, which supports earlier findings from the laboratory, and indicates that Cu is homeostatically regulated in fish (cf. Benoit 1975, Brungs et al. 1975).

Cadmium showed a strict linear concentration dependency in fish liver, which indicates that net uptake was to minor extent effected by variations in humic content.

Lead in perch liver revealed a weak concentration dependency and a low bioconcentration factor. Low bioavailability of Pb correlates with small dialysability and filterability, and large accumulation factors in sediment.

The particulate/colloidal "nature" of Pb is supported by Asellus exhibiting a steep concentration dependency; of simlar type as was found in the sediments. The colloidal "nature" of Pb is further supported by the concentration dependency in transplanted moss, being lower in dialysis tubes than in net-boxes and filter chambers.

Thus, before dismissing total metals or filterable fractions as

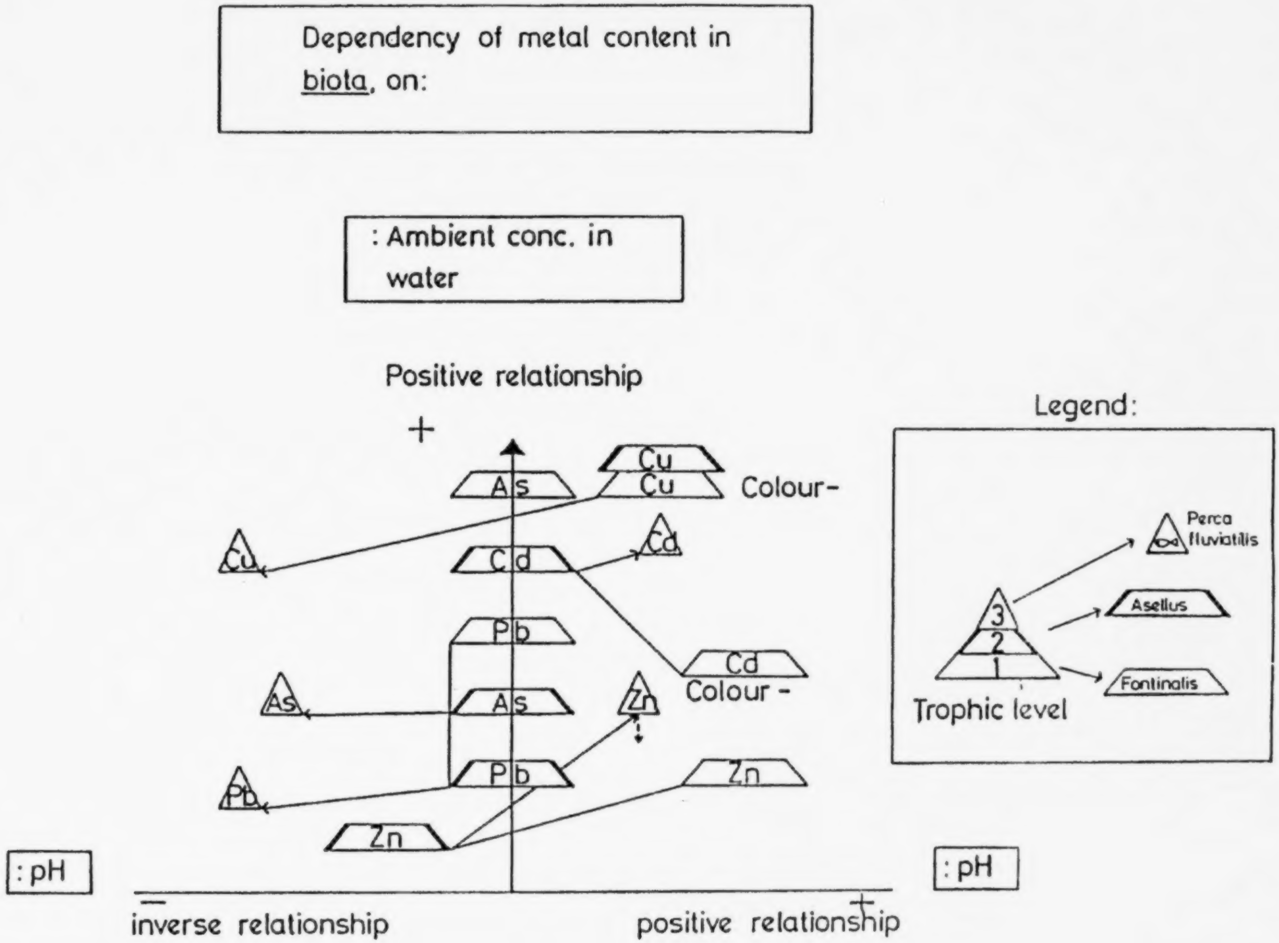
Dependency of metal content in biota, on:
: Ambient conc. in water
Positive relationship
+
Cu
Cu
Colour-
As
Cu
Cd
Cd
Pb
Cd
Colour -
As
As
Zn
Pb
Pb
Zn
Zn
: pH
: pH
−
inverse relationship
+
positive relationship
Legend:
Perca fluviatilis
Asellus
3
2
1
Fontinalis
Trophic level

Figure 2.

are biologically inactive in organisms such as Fontinalis and Asellus; as well as in mussels, where an uptake of particulate Pb by pinocytosis has been demonstrated.

Copper and arsenic in transplanted moss were stronger correlated with dialyzable metals than with other fractions and may have implications for other organisms as well.

The metal content in Water Hog-louse showed no clear-cut correlation with pH. In aquatic moss the bioconcentration factor of Zn, Ni, Co and Cd increased with increasing pH. Cadmium revealed a similar tendency in fish, probably as a result of H+-metal interactions at biological surfaces (Campbell & Stokes 1985).

On the other hand, the bioconcentration factor of Cu, Pb and As in fish liver/muscle, increased at pH less than 6. It indicates that the effect of pH on chemical speciation probably is stronger than, and is out-balancing the interactions at biological surfaces.

CONCLUSIONS

The response of biota to ambient concentrations, pH, and chemical speciation, is partly variable and a function of species and/or trophic level. One possible explaination, being differenences in exposure routes of metals. Other confounding factors are interactions between hydrogen ions and metals at biological surfaces, and difficulties to separate particulate and adsorbed, from truely bioaccumulated metals.

In conclusion, a broad definition of biological availability is needed, which includes all trophic levels.

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WORKSHOP PROCEEDINGS/ COMPTE RENDUS DU COLLOQUE

The proceedings of the Annual Aquatic Toxicity Workshops have been published as a series of technical reports listed below. Copies of recent Proceedings are available from Dr. A. Niimi, Continuity Chairman, Aquatic Toxicity Workshop, Bayfield Institute, Canada Centre for Inland Waters, Burlington, Ontario, Canada, L7R 4A6 (416-336-4868). Copies of most Proceedings are available for a charge from Micromedia Limited, 165 Hotel de Ville, Place du Portage, Hull, Quebec, J8X 3X2, (819-770-9928). Their catalogue numbers (MLCN) are listed where applicable.

Proceedings of the Sixteenth Annual Aquatic Toxicity Workshop: November 6-8, 1989, Winnipeg, Manitoba. Edited by S.L. Leonhard. Can. Tech. Rep. Fish. Aquat. Sci. (In Preparation).

Proceedings of the Fifteenth Annual Aquatic Toxicity Workshop: November 28-30, 1988, Montreal, Quebec. Edited by R. Van Coillie, A. Niimi, A. Champoux and G. Joubert. Can. Tech. Rep. Fish. Aquat. Sci. 1714: 244 p. (MCLN: 90-01805).

Proceedings of the Fourteenth Annual Aquatic Toxicity Workshop: November 2-4, 1987, Toronto, Ontario. Edited by A.J. Niimi and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1607: 201 p. (MLCN: 88-04587).

Proceedings of the Thirteenth Annual Aquatic Toxicity Workshop: November 12-14, 1986, Moncton, New Brunswick. Edited by J.S.S. Lakshminarayana. Can. Tech. Rep. Fish. Aquat. Sci. 1575: 178 p. (MLCN:88-01709).

Proceedings of the Twelfth Annual Aquatic Toxicity Workshop: November 5-8, 1985, Thunder Bay, Ontario. Edited by G.W. Ozburn. Can. Tech. Rep. Fish. Aquat. Sci. 1462: 229 p. (MLCN: 86-5828).

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Proceedings of the Tenth Annual Aquatic Toxicity Workshop: November 7-10, 1983, Halifax, Nova Scotia. Edited by P.G. Wells and R.F. Addison. Can. Tech. Rep. Fish. Aquat. Sci. 1368: 475 p. (MLCN: 86-1103).

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Proceedings of the Eighth Annual Aquatic Toxicity Workshop: November 2-4, 1981, Guelph, Ontario. Edited by N.K. Kaushik and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 255 p. (MLCN: 83-2515).

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Proceedings of the Second Annual Aquatic Toxicity Workshop, November 4-5, 1975. Rexdale, Ontario. Edited by G.R. Craig. Ontario Ministry of the Environment.

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